South Western Journal of
Horticulture, Biology and Environment
P-Issn: 2067- 9874, E-Issn: 2068-7958

Vol.15, No.1, 2024
pp.19-38
Art.no. e24102

OPTIMIZATION OF *in vitro* PROTOCOL FOR SURVIVAL AND REGENERATION OF GAMMA-INDUCED SCALPS IN *Musa acuminata* cv. Calcutta-4

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ABSTRACT. *This study aimed to investigate the impact of varying gamma irradiation doses on banana scalps' survival and regeneration potential, specifically focusing on their interaction with different culture media components. Banana scalps, derived from a selected cultivar (Calcutta-4), were exposed to gamma irradiation doses ranging from 10 Gy to 70 Gy. Following irradiation, the scalps were transferred to fresh culture media, and their survival rates and regeneration potential were assessed. The findings revealed a clear inverse relationship between radiation dose and scalp survival. Lower doses of 10 Gy and 20 Gy exhibited higher survival rates when cultured on a modified MS medium. In comparison, higher doses, such as 50 Gy and 60 Gy, led to a significant decrease in survival. The optimal gamma irradiation dose for both scalp survival and successful regeneration fell within the range of 30 Gy to 40 Gy when using a medium supplemented with lower concentrations of BAP and Ads (1.5 mg/l to 2.0 mg/l BAP + 200 mg/l to 300 mg/l Ads). Furthermore, the study demonstrated that the surviving irradiated scalps exhibited a high potential for shoot regeneration when cultured on an MS medium with 3.0 mg/l BAP + 400 mg/l Ads. Gamma irradiation facilitated the initiation of new shoots, and the specific concentration of plant growth regulators and media composition influenced the response. These findings have significant implications for optimizing tissue culture techniques and improving crop production in banana cultivars and are crucial for advancing banana farming practices.*

KEYWORDS: *Gamma irradiation, micropropagation, Musa acuminata, regeneration, scalp survival.*

ABBREVIATIONS: *BAP: 6-Benzylaminopurine; IAA: Indole-3-acetic acid; IBA: Indole-3-butyric acid; MS: Murashige and Skoog (1962) medium; Ads: Adenine sulfate.*

INTRODUCTION

Bananas, a crucial crop in Bangladesh and global agriculture, are a prominent staple food and source of income (FAO 2021). Smallholder farmers predominantly cultivate bananas, and local entrepreneurs play a vital role in the trade and distribution of this fruit within the local and international markets, contributing significantly to the economy (Frison et al. 1997, FAO 2017, Porter & Howard 2018, Tripathi et al. 2020).

Beyond its economic importance, bananas offer substantial health benefits. They are a rich source of essential nutrients and dietary fiber, making them a valuable component of a nutritious diet (Kumari et al. 2023, Ranjha et al. 2022). The cultivation and trade of bananas have become a cornerstone of livelihoods, supporting farmers and various actors within the supply chain (Mohapatra et al. 2010, Robinson & Galán Saúco 2010).

However, the genetic uniformity resulting from the common use of clonal propagation methods, such as tissue culture and suckers, makes bananas highly susceptible to pests, diseases, and environmental stress (Robinson & Galán Sauco 2010, Jain & Veilleux 2010, Jones 2009, Ploetz 2015).

Calcutta-4, classified under the genomic group AA, is a seeded banana accession belonging to *Musa acuminata* (Ganapathi et al. 2008, Simmonds & Shepherd 1955, Bakry et al. 2009). This particular cultivar, renowned for its robust resistance to various plant diseases, has played a pivotal role in breeding programs (Bakry et al. 2009, Singh et al. 2011). It exhibits remarkable resistance to black leaf streaks, Sigatoka leaf spots, and various races of Fusarium Wilt and displays partial resistance to nematodes like *Radopholus similis* (Robinson 1982).

Gamma irradiation and induced mutagenesis

Gamma irradiation is a powerful tool for inducing mutagenesis in plants. It involves exposing plant tissues to ionizing gamma rays, which can cause

genetic mutations by damaging DNA (Hasim et al. 2021, Kodym et al. 2012, Mba 2013, Pachakkil & Salim 2017). In bananas, this mutagenic treatment can lead to genetic variations, providing a valuable resource for breeding programs and crop improvement (Hasim et al. 2021, Peraza-Echeverria et al. 2012, Bakry et al. 2009)

Induced mutagenesis via gamma irradiation is a controlled process that allows researchers to introduce genetic changes without altering the overall genomic structure (Hasim et al. 2021). The extent of genetic variation can be regulated by adjusting the irradiation dose and duration (Beyaz & Yildiz 2017, Peraza-Echeverria et al. 2012, FAO/IAEA 2013, Mba 2013).

Genetic variability introduced through gamma irradiation can result in plants with improved resistance to diseases, such as Panama disease and black Sigatoka (Kumar et al. 2015, Peraza-Echeverria et al. 2012, Jain & Veilleux 2010). Mutagenesis can lead to the development of banana varieties with improved yield, taste, and nutritional content, making them more appealing to consumers (Ortiz & Swennen 2014, D'Hont et al. 2012)

Variants generated through irradiation may exhibit enhanced tolerance to environmental stress factors like drought and salinity (Bakry et al. 2009). Diverse banana cultivars are vital for long-term sustainability of the crop, as they can mitigate the risks associated with monoculture and the emergence of new pests and diseases (Bakry et al. 2009, Jain & Veilleux 2010).

While gamma irradiation offers significant potential for enhancing banana genetic diversity, there are several important considerations: proper safety protocols must be followed to protect researchers and the environment from radiation exposure (IAEA 2015, FAO/IAEA 2013). Identifying and selecting desirable mutant lines can be a time-consuming and labor-intensive process (Singh et al. 2011, Pachakkil & Salim 2017). In some regions, induced mutagenesis may require regulatory approval, as it involves genetic modification (IAEA 2019, Bakry et al. 2009). The study presented in this article investigates the impact of gamma irradiation on banana shoot tip culture, focusing on the roles of different plant growth regulators and their concentrations, particularly ads (adsorbents), in the induction of shoot bud proliferation and subsequent scalp regeneration.

Scalp production *in vitro*

In vitro propagation techniques have played a pivotal role in advancing the production of banana plants (Kishor et al. 2017, Teisson & Côte 1997),

particularly in addressing issues related to disease management, genetic diversity, and mass multiplication (Rajoriya et al. 2018, Iqbal et al. 2013, Kaçar & Faber 2012).

Scalp production in vitro is one such technique that holds promise for banana propagation, allowing for the rapid generation of disease-free and genetically identical banana plants. Scalping is a micropropagation method that isolates small meristematic tissue pieces from a banana plant's shoot tip or corm and cultures them under sterile conditions on a nutrient-rich medium. This technique enables the generation of multiple plantlets from a single explant, ensuring genetic uniformity (Mahdi et al. 2014, Hasan et al. 2020, Peraza-Echeverría et al. 2012, Heslop-Harrison & Schwarzacher 2007)

Scalp production *in vitro* is particularly advantageous for producing disease-free banana plantlets. The technique effectively eliminates the transmission of various viral and bacterial pathogens, such as Banana Streak Virus (BSV) and Banana Bunchy Top Virus (BBTV) (Brown et al. 2017). Scalping allows for the rapid multiplication of banana plants, resulting in a high number of plantlets within a short time frame. This feature is essential for addressing the increasing demand for banana planting material (Heslop-Harrison & Schwarzacher 2007, Peraza-Echeverría et al. 2012). As all plantlets are derived from a single source, they are genetically identical, ensuring uniformity in terms of traits and characteristics. Scalp production *in vitro* is instrumental in conserving elite banana varieties by maintaining their genetic purity (Peraza-Echeverría et al. 2012).

Gamma irradiation in *in vitro* scalp culture

Gamma irradiation is a technique that involves exposing plant tissues and scalp-cultured banana explants to ionizing gamma rays. This exposure induces mutations by causing DNA damage, resulting in genetic variability (Kulkarni et al. 2007, Kodym et al. 2012, Mba 2013, Wang et al. 2018). When applied to in vitro scalp cultures, gamma irradiation provides a controlled and precise method for introducing genetic changes without altering the overall genomic structure, thereby allowing the development of new traits and characteristics in banana plants (Vuylsteke & Swennen 1992, Wang et al. 2018).

Gamma irradiation can lead to genetic variations in banana plants, thereby increasing the genetic diversity within a cultivar (Gowda et al.

2010). This diversity can be valuable for breeding programs and crop improvement. Mutagenesis induced by gamma irradiation can result in the development of banana plants with novel and desirable traits, such as disease resistance, improved fruit quality, or stress tolerance (Gowda et al. 2010). The controlled mutagenesis achieved through gamma irradiation can accelerate the evolution of banana varieties, enabling researchers to develop improved cultivars more rapidly (Justine et al. 2022). The success of gamma irradiation in *in vitro* scalp culture depends on optimizing the irradiation dose and duration to balance the induction of genetic variations with plant viability and survival (Kodym et al. 2012, Wang et al. 2018).

MATERIAL AND METHODS

In this study, shoot tip explants of the banana cultivar Calcutta-4 were collected from field-grown sword suckers at the Atomic Energy Research Establishment in Savar, Dhaka, Bangladesh. The shoot regeneration and micropropagation were conducted using Murashige and Skoog (MS) medium with various concentrations and combinations of growth regulators.

Scalp formation and gamma irradiation

Shoots were treated with varying concentrations of BAP and Ads, causing them to become short and spread. Meristems were exposed by removing leaves and blackish basal tissues. Since the explants used in the study consisted of meristems, the resulting in vitro cultures are inherently free from viruses, bacteria, and fungi, ensuring that the cultures are healthy (Vuylsteke & Swennen 1992). This method of using meristematic tissue is crucial as it minimizes the risk of pathogen transmission, which is often a significant concern in plant propagation and tissue culture practices. The meristematic regions are less likely to be infected by pathogens, providing a reliable source of disease-free plant material (Lassois et al. 2013). After seven subcultures, small white meristems appeared at the shoot's base. These tiny meristems were then isolated and placed in a growth medium. Highly proliferating scalps with fleshy bulbous structures containing tiny meristems were obtained within a month. Scalp induction responses depended on the interaction between BAP concentration and the banana cultivar used.

Gamma irradiation was applied to the scalps of the Calcutta-4 banana cultivar with doses ranging from 10 Gy to 70 Gy using a cobalt-60 irradiator at 20 Gy. Following irradiation, the scalps were transferred to sterile fresh media. Sixty percent of the cultured jars were irradiated with various doses and immediately subcultured. Meanwhile, 40% were converted to shoots without radiation by

culturing with lower concentrations of BAP and Ads in MS media.

***In vitro* shoot formation from the scalps**

After each irradiation, the cultures were immediately subcultured on shoot proliferation media. Irradiated scalps were excised and placed on fresh media for conversion into plantlets using lower concentrations of BAP (ranging from 1.5 to 5.0 mg/l) and Ads (400 mg/l) in MS media. Subcultures were performed at 30-day intervals, resulting in the growth of numerous shoots by increasing the BAP concentration.

Root induction from shoots

When the shoots reached lengths of about 4-5 cm with 3-4 well-developed leaves, they were aseptically removed from the culture tubes and cultured on freshly prepared medium supplemented with various concentrations of Indole-3-butyric acid (IBA) and Indole-3-acetic acid (IAA) ranging from 0.5 to 2.5 mg/l to induce root formation.

Transplantation

After developing sufficient root systems, the regenerated plantlets were ready for transfer to soil. The plantlets were carefully washed to remove agar from their roots and transplanted into small polythene bags containing 500 grams of a soil mixture. This mixture consisted of soil, sand, and compost in a 2:1:1 ratio. The pot plantlets were covered with polythene bags to maintain high humidity and sprayed with water every 24 hours. Eventually, the plantlets were transferred to the experimental field.

Data analysis

The plant samples were chosen randomly and evenly distributed, with each experiment replicated at least three times. The results are expressed as mean values along with their respective standard deviations. Rigorous statistical analysis was conducted on the data obtained in this study. One-way analysis of variance (ANOVA) was utilized to evaluate variations among different treatments. Significance between any two means was determined at a selected probability level of $p = 0.05$, employing Duncan's Multiple Range Test (DMRT). The statistical software used for these analyses was SPSS Statistic 25.0.

RESULTS

In this study, shoot tip explants from banana cultivar Calcutta-4 were used to investigate the impact of various plant growth regulators on shoot

regeneration, multiplication, scalp formation, and the potential for gamma irradiation in shoot regeneration and rooting.

Shoot-bud induction

The explants were cultured on MS media with different combinations and concentrations of BAP and Kin, BAP and 2ip, and BAP and Ads. The most effective formulation for shoot-bud induction was observed with 3 mg/l BAP + 0.4 mg/l Kin, resulting in a 58% response rate. The highest number of shoots per explant (1.90 ± 0.21) and the longest shoots (3.31 ± 0.19 cm) were obtained with 3.0 mg/l BAP + 0.4 mg/l Kin (Table 1).

Table 1. Effect of different concentrations of hormone with MS media on *in vitro* shoot tip culture at 120 days after inoculation.

Concentrations of hormones	% shoot tip responded to shoot induction	No. of shoots/explant Mean ± SE	Length of shoots (cm) Mean ± SE
BAP + Kin (mg/l)			
1.5 + 0.1	25±0.33^{a}	1.20 ± 0.14^{a}	1.50 ± 0.24^{a}
2.0 + 0.2	47±0.55^{b}	1.50 ± 0.23^{b}	2.53 ± 0.37^{b}
2.5 + 0.3	50±0.69^{c}	1.45 ± 0.32^{bc}	2.80 ± 0.32^{b}
3.0 + 0.3	58±0.79^{d}	1.60± 0.24^{bc}	2.95 ± 0.32^{c}
3.0 + 0.4	62±0.53^{e}	1.90 ± 0.13^{c}	3.31 ± 0.19^{d}
3.5 + 0.5	52±0.39^{f}	1.58 ± 0.22^{d}	3.14 ± 0.12^{d}
BAP + 2ip (mg/l)			
1.5 + 0.1	20±0.78^{a}	1.00 ± 0.28^{a}	1.88 ± 0.41^{a}
2.0 + 0.2	44±0.88^{b}	1.30 ± 0.12^{b}	1.86 ± 0.32^{a}
2.5 + 0.3	45±0.98^{c}	1.25 ± 0.19^{b}	1.97 ± 0.35^{a}
3.0 + 0.4	60±0.79^{d}	2.63 ± 0.21^{d}	3.10 ± 0.23^{c}
3.5 + 0.5	46±1.12^{b}	1.50 ± 0.31^{c}	2.84 ± 0.26^{b}
BAP + Ads (mg/l)			
1.5 + 50	40±0.92^{a}	2.20 ± 0.16^{a}	2.68 ± 0.17^{a}
2.0 + 100	42±0.78^{b}	2.60 ± 0.23^{b}	2.75 ± 0.29^{a}
2.5 + 150	60±0.88^{c}	2.66 ± 0.41^{b}	2.92 ± 0.35^{b}
3.0 + 200	63±0.54^{c}	3.70 ± 0.33^{c}	3.32 ± 0.19^{c}
3.5 + 250	77±1.10^{d}	6.50 ± 0.21^{d}	3.57 ± 0.31^{d}

*Means with no letter in common are significantly different. *(DMRT test; $p \leq 0.05$)*

Similarly, 3.0 mg/l BAP + 0.4 mg/l 2ip was the best combination for shoot-bud proliferation with the highest response rate. The maximum number of shoots (2.63 ± 0.21) and the longest shoots (3.10 ± 0.23 cm) were recorded. The combination of 3.5 mg/l BAP + 250 mg/l Ads resulted in a 77% response rate for shoot formation when using BAP and Ads. This combination also yielded the highest number of shoots per explant (6.50 ± 0.21) and the longest shoots (3.57 ± 0.31 cm) (Table 1).

Scalp induction

Scalp induction was successful when higher concentrations of BAP and Ads were used. The best combination for shoot induction was 6 mg/l BAP + 400 mg/l Ads, while the best for scalp formation was 10 mg/l BAP + 600 mg/l Ads, both of which were most effective. This cultivar exhibited the highest percentages of scalp formation (100%) and the maximum number of scalps per explant (6.90 ± 0.42) (Table 2, Figure 2).

Table 2. Effect of different concentrations of BAP and Ads with MS media on scalp formation, No. of scalps per explants, and Length of shoots from shoot tip explants at 210 days after inoculation.

Concentrations of hormones	% scalp induced/ explants	No. of scalps / explants Mean ± SE	No. of scalps /explants Mean ± SE	Length of Shoots (cm) Mean ± SE
BAP + Ads (mg/l)				
2.0 + 200	0	0	3.60 ± 0.45^{c}	3.70 ± 0.65^{c}
4.0 + 300	0	0	4.20 ± 0.23^{d}	4.10 ± 0.32^{d}
6.0 + 400	67 ± 1.92^{a}	3.20 ± 0.12^{a}	6.10 ± 0.53^{f}	6.61 ± 0.32^{e}
8.0 + 500	80 ± 2.48^{b}	5.50 ± 0.37^{d}	5.23 ± 0.27^{e}	3.82 ± 0.45^{d}
10.0 + 600	100 ± 2.68^{d}	6.33 ± 0.19^{b}	3.12 ± 0.13^{b}	2.10 ± 0.24^{b}
12.0 + 700	89 ± 2.40^{c}	4.12 ± 0.32^{c}	1.80 ± 0.29^{a}	1.94 ± 0.31^{a}

*Means with no letter in common are significantly different. *(DMRT test; $p \leq 0.05$)*

Response to gamma irradiation

The radio-sensitivity study assessed scalp survival in response to gamma irradiation 30 days after exposure. Scalps were irradiated with doses ranging from 10 Gy to 70 Gy and then transferred to a fresh medium for growth. It was observed that scalp survival percentage decreased with

increasing gamma irradiation doses. Lower doses (10 Gy and 20 Gy) enhanced scalp survivability compared to higher doses (50 Gy and 60 Gy). The optimum survival dose was 30-40 Gy, with 50% of irradiated scalps surviving at this gamma irradiation level. The lethal dose for 100% scalp mortality was determined at 70 Gy, while a lethal dose for 50% was found at 40 Gy (Figure 1, Figure 3 A-D).

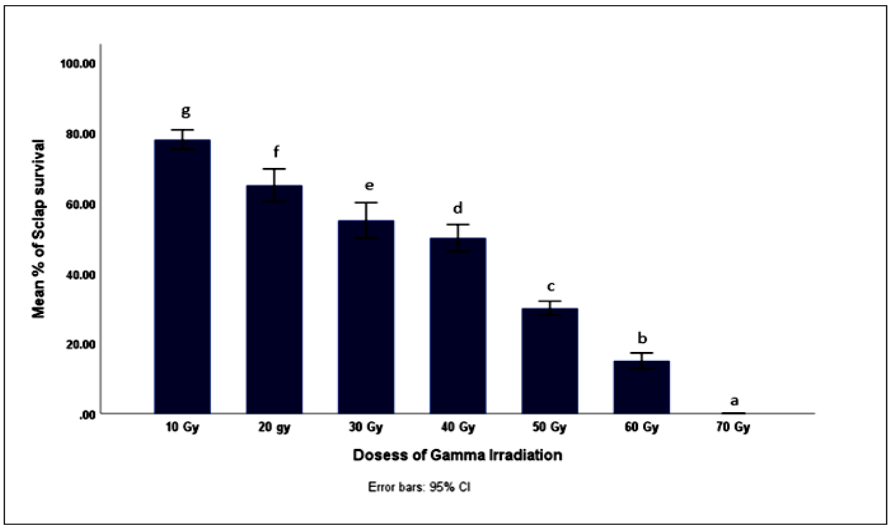

Figure 1. Gamma Irradiation doses and the percentage of survival of scalp (*Means with no letter in common are significantly different. (DMRT test; $p \leq 0.05$))

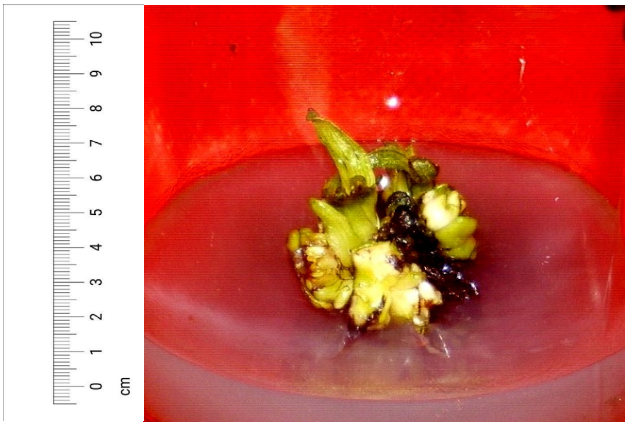

Figure 2. *In vitro* scalp induction from shoot tip explants 210 days after inoculation before Gamma irradiation

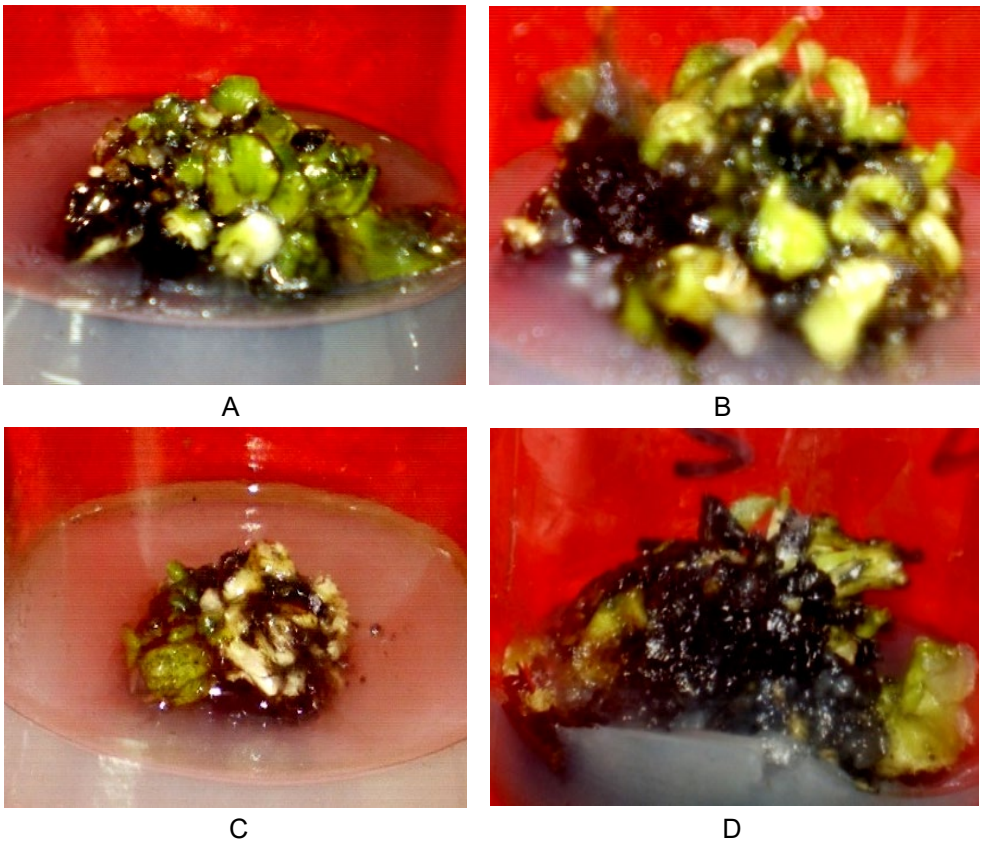

Figure 3. Radio-sensitivity response to scalps at 30 days after treated with different doses of gamma irradiation: A-30*Gy,* B-40*Gy,* C-50*Gy,* and D-70*Gy*.

Shoot induction from the scalp

Scalps were cultured on MS media with lower concentrations of BAP and Ads for shoot induction. The maximum number of scalps inducing multiple shoots was observed in (8.10 ± 0.44) with hormonal concentrations of 3.0 mg/l BAP + 400 mg/l Ads. The longest shoots were found in Calcutta-4 (4.30 ± 0.19 cm) on MS media containing 4.0 mg/l BAP + 400 mg/l Ads. exhibited a 100% shoot induction rate, with 8.10 ± 0.44 shoots per explant and 4.30 ± 0.19 cm shoot length. Dhed'a et al. (1991) expressed that the scalp results from converted shoot tips into compact clumps of meristematic buds. Reduction of BAP to a lower concentration had triggered prolific shoot regeneration from scalps of cv. Calcutta 4 (Table 3).

Table 3. Effect of gamma irradiation on *in vitro* shoot regeneration from scalps using different concentrations of BAP and 400 mg/l Ads in MS supplemented media of five banana cultivars at 120 days after inoculation.

Concentrations of hormones	% scalps induced shoots	No. of shoot/scalp Mean ± SE	Length of shoot (cm) Mean ± SE
BAP + 400 mg/l Ads			
1.5 + 400	63± 1.18[a]	4.79 ± 0.23[a]	2.20 ± 0.41[a]
2.0 + 400	74± 1.46[c]	5.20 ± 0.19[c]	2.67 ± 0.38[b]
3.0 + 400	86± 1.31[d]	6.68 ± 0.41[d]	3.14 ± 0.31[c]
4.0 + 400	78± 1.19[c]	6.10 ± 0.43[b]	3.25 ± 0.43[d]
5.0 + 400	72± 1.24[b]	5.45 ± 0.44[c]	2.54 ± 0.44[b]

*Means with no letter in common are significantly different. *(DMRT test; $p \leq 0.05$)*

Shoot proliferation from gamma-irradiated scalps

In the study of shoot proliferation from gamma-irradiated scalps, the LD50 was determined at 40 Gy. Cultivars responded to shoot induction across all media combinations. The maximum shoot induction from scalps (86%) was observed on MS medium containing 3.0 mg/l BAP + 400 mg/l Ads. The highest number of multiple shoots was seen (6.68 ± 0.41), with a maximum shoot length of 3.25 ± 0.43 cm on MS media with 4.0 mg/l BAP + 400 mg/l Ads. The lowest percentage of shoot induction (63%) was noted on MS media with 1.5 mg/l BAP + 400 mg/l Ads, resulting in the minimum number of multiple shoots (4.79 ± 0.23) (Table 3, Figure 4).

Figure 4. *In vitro* shoot formation from Gamma eradiated scalp on 120 days after inoculation.

Root induction

Root induction was observed for various media combinations. Calcutta-4 displayed the highest root induction percentage (100%) on ½ strength MS media containing 2.0 mg/l IBA and the maximum number of roots (6.20 ± 0.24). The lowest rooting response was recorded with 0.5 mg/l IBA in ½ strength MS-supplemented media, resulting in the lowest number of roots (4.12 ± 0.38) (Table 4, Figure 5 A).

Table 4. Effect of different concentrations of hormones with ½ strength of MS media on *in vitro* root induction from scalp-derived shoots at 40 days after inoculation.

Concentrations of hormones	% shoot responded to root induction	No. of root/explants Mean ±SE	Length of root (cm) Mean ± SE
IBA (mg/l)			
0.5	75± 0.98^{a}	4.12 ± 0.38^{a}	3.39 ± 0.55^{a}
1.0	89± 1.18^{b}	4.70 ± 0.44^{b}	3.55 ± 0.28^{a}
1.5	92± 1.33^{b}	5.62 ± 0.33^{c}	4.62 ± 0.43^{c}
2.0	100± 1.78^{c}	6.20 ± 0.24^{d}	5.32 ± 0.26^{d}
2.5	88± 1.38^{b}	5.36 ± 0.65^{c}	4.22 ± 0.32^{b}
IAA (mg/l)			
0.5	58± 0.72^{a}	3.40 ± 0.33^{a}	2.51 ± 0.48^{a}
1.0	60± 0.88^{a}	3.93 ± 0.42^{b}	3.10 ± 0.33^{b}
1.5	74± 1.12^{b}	4.75 ± 0.42^{c}	4.23 ± 0.42^{c}
2.0	85± 1.98^{c}	5.10 ± 0.42^{d}	4.30 ± 0.37^{c}
2.5	72± 1.08^{b}	3.98 ± 0.29^{b}	3.14 ± 0.31^{b}
NAA (mg/l)			
0.5	58± 0.72^{a}	3.65 ± 0.27^{a}	2.88 ± 0.32^{a}
1.0	72± 0.72^{a}	4.20 ± 032^{b}	3.30 ± 0.21^{b}
1.5	90± 0.72^{a}	4.90 ± 0.45^{d}	3.82 ± 0.44^{d}
2.0	82± 0.72^{a}	4.58 ± 0.41^{c}	3.68 ± 0.23^{c}
2.5	76± 0.72^{a}	4.12 ± 0.29^{b}	3.40± 0.23^{b}

*Means with no letter in common are significantly different. *(DMRT test; $p \leq 0.05$)*

In another set of experiments, scalp-derived shoots were cultured on ½ strength MS media supplemented with different concentrations of IAA. The maximum root induction percentage (85%) was observed in Calcutta-4 on

media containing 2.0 mg/l IAA. In this formulation, the maximum number of roots (5.10 ± 0.18) and the longest roots were observed. *In vitro* irradiated scalp-derived shoots were cultured on ½ strength MS media with various concentrations of IAA for root induction, showing the maximum root induction percentage (75%) on the same media composition (Table 4).

Transplantation

90% of the plants survived in small polythene bags containing 500 grams of a soil mixture composed of soil, sand, and compost in a 2:1:1 ratio. Plantlets with well-developed roots were successfully transferred to the field after undergoing thorough ex-vitro acclimatization procedures (Figure 5 C and D).

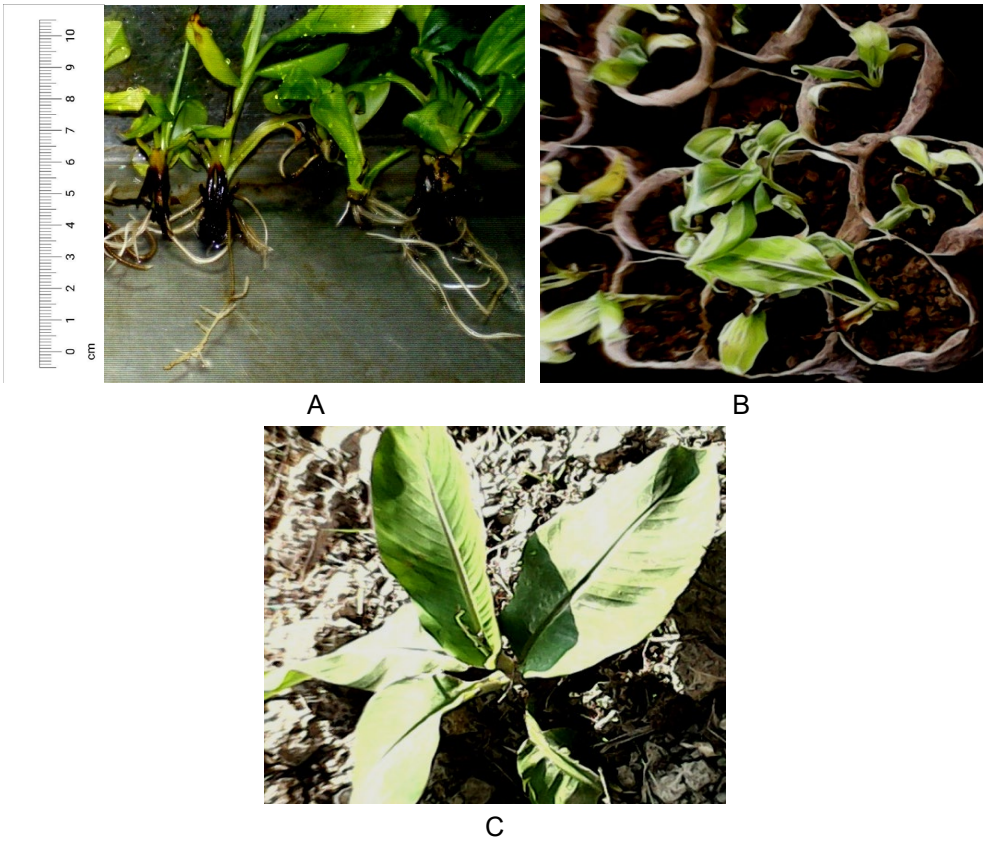

Figure 5. A - Plantlet with root; B - Plantlet in potting mix for acclimatization; C - Planting in the field

DISCUSSION

In our study, the most effective formulation for shoot-bud induction was observed with 3 mg/l BAP + 0.4 mg/l Kin, a similar result was obtained by Rajoriya et al. (2018), Bower (1982), Iqbal et al. (2013) who employed MS media supplemented with 5 mg/l BAP + 2 mg/l IAA and 5 mg/l BAP + 1 mg/l IAA respectively for shoot tip culture, resulting in the production of multiple shoots in banana. For shoot-bud proliferation, 3.0 mg/l BAP + 0.4 mg/l 2ip was found to be the best combination, displaying the highest response rate in our study, which is similar to the study of Rajeevan & Pandey (1986), who observed that shoot-tip explants successfully formed multiple shoots when cultured in media containing kin and NAA, as well as BAP and NAA.

However, the investigation also delves into the effects of gamma irradiation on banana scalps, an essential aspect for inducing mutations and improving crop productivity (Abdulhafiz et al. 2018, Sales et al. 2013, Pestana et al. 2011, Spencer et al. 2018). The findings reveal an inverse relationship between radiation dose and scalp survival, with lower doses (10 Gy and 20 Gy) enhancing survivability compared to higher doses (50 Gy and 60 Gy). The determination of the optimal radiation dose is necessary for the efficient induction of mutagenesis by gamma radiation (GR), which is the dose that reduces half of the population (median lethal dose, LD50). Different plant tissues (seed, meristem, callus, scalps, etc.), stage of development, and moisture content, among other parameters, require different doses (Pachakkil & Salim 2017, Sales et al. 2013, Álvarez et al. 2019). Therefore, efficient dosing prevents radio inhibition, affecting growth promoters and, eventually, tissue destruction (Surakshitha & Soorianathasundaram 2017). High radiation doses can also alter regenerative capacity, cause malformation, and even destruction of plant tissues (Chakravarty & Sen 2001). Radiosensitivity assays allow determining the appropriate radiation dose to induce the highest mutation rate with the most negligible effects on the gene complex (Spencer et al. 2018).

Scalps exhibit a remarkable proliferative capacity and serve as valuable sources for embryogenesis (Dhed`a et al. 1991, Strosse et al. 2003, 2006). Sholi et al. (2009) proposed that various *Musa* genotypes and cultivars exhibit distinct plant growth regulator (BAP) level requirements to induce scalp formation. The interplay between BAP concentration and specific

cultivars influenced the responses to scalp induction. It's important to note that bananas have been recognized as a challenging material for *in vitro* plant regeneration (Strosse et al. 2003, Venkatachalam et al. 2006).

The study emphasizes the importance of optimizing the concentration of plant growth regulators, specifically BAP and Ads, for the induction of shoot buds from scalps. The gamma irradiation process enhances the initiation of new shoots, and the response is influenced by specific concentrations of these growth regulators and the overall media composition; similar findings were presented by Mahdi et al. (2014), Iqbal et al. (2013) and Pestana et al. (2011).

The shoot tip culture system in banana and plantain has been employed for inducing mutations, as noted by Novak (1992) and Kulkarni et al. (1999). In vegetatively propagated plant species, *in vitro* propagation via shoot apices can generate a large population of shoots before and after radiation treatment. The percentage of shoot survival decreased as the irradiation dose increased, indicating an inverse relationship between these two factors. Low-dose gamma irradiation has been utilized in tissue culture to stimulate growth. Multiple shoot cultures were exposed to gamma rays at doses of 0, 5, 10, and 30 Gy, with a radiation rate of 20 Gy per minute, and subsequently subcultured onto MS (Murashige & Skoog 1962) media supplemented with 2 mg/l benzyl adenine and 30 mg/l adenine sulfate, as described by Ganapathy et al. (1995).

The investigation concludes with insights into the root initiation process for the regenerated shoots. Selecting auxins, specifically IBA, is crucial in achieving successful root induction Suman et al. (2013). These findings were similar to those of Sarwoar et al. (2003) and Rajoriya et al. (2018), who reported that root induction decreased gradually with an increase in the radiation dose.

After successful shoot regeneration from scalps in a controlled in vitro environment, the next critical step is transplanting these regenerated shoots to the field. In the present study 90% of the plants survived in small polythene bags containing 500 grams of a soil mixture composed of soil, sand, and compost in a 2:1:1 ratio. Various soil mixtures, containing different ratios of vermiculite, peat moss, perlite, sand, and vermicompost, have been utilized for the successful hardening of banana cultivars (Robinson & Galán Sáuco 2009, Safarpour et al. 2017, Chamling & Bhowmick 2021). This transplantation process aims to acclimate the

banana plants to natural conditions, allowing them to adapt and thrive in the external environment (Robinson & Galán Sáuco 2009). Additionally, sand and farmyard manure (FYM) have been used to acclimate and harden banana cultivars Malbhog and B.B. Battisa, achieving 95% and 80% survival rates, respectively (Suman et al. 2013).

CONCLUSIONS

The radiation dose inversely influenced scalp survivability, with lower doses (10 Gy and 20 Gy) promoting higher survival rates. However, scalp survival significantly declined as the radiation dose increased (50 Gy and 60 Gy).

These findings are of utmost importance for optimizing tissue culture techniques and enhancing crop production in banana cultivars. The ability to fine-tune the delicate balance between scalp survival and regeneration, contingent on utilizing specific culture media, holds significant promise for micropropagation and the overall improvement of banana farming practices.

This research provides valuable insights into the potential benefits of gamma irradiation in enhancing banana crop adaptability and productivity. By harnessing the scalp's regenerative power, banana farming can gain a novel approach to bolster its sustainability and yield.

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