Effects of ^{60}Co gamma-ray irradiation on *in vitro* shoot regeneration and mutation induction in sweet potato *(Ipomoea batatas)*

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ABSTRACT

Sweet potato (*Ipomoea batatas*) is a vital crop used for various purposes, including food, feed, and raw materials. However, its production in Vietnam is being adversely affected by climate change, including changes in rainfall patterns, droughts, and saltwater intrusion. To address these challenges, improving crop diversity is essential to enhance yield, nutritional content, and stress tolerance. This study investigated the effects of ^{60}Co gamma-ray irradiation on shoot regeneration from sweet potato shoot tips and nodal segments, followed by the detection of mutations in newly regenerated shoots. The experimental procedures included optimizing the *in vitro* culture medium and assessing the impact of varying doses of gamma irradiation on survival, mutation formation, and subsequent recovery across generations. The Murashige and Skoog (MS) medium, supplemented with 2 mg/L 6-benzylaminopurine (BAP) and 0.2 mg/L indole-3-butyric acid (IBA), was found to be optimal for shoot regeneration. Gamma irradiation doses ranging from 0 to 90 Gy were applied. The results showed that the lethal dose 50 (LD_{50}) was 59.3 Gy for nodal segments and 80 Gy for shoot tips. The highest regeneration and survival rates were observed at lower radiation doses (30 Gy), while a 50 Gy irradiation dose resulted in a morphological variation frequency of 8.97%, with the most diversified types of morphological variances. Numerous morphological variances including split leaves, chlorophyll loss, light leaf veins, wavy leaves, stems without apical meristems, and abnormal root proliferation, were observed at different rates after irradiation with 30, 50, or 70 Gy. Genetic analysis using Start Codon Targeted (SCoT) markers confirmed genetic differences between the variants and the mother plant, indicating the effectiveness of this approach in enhancing genetic diversity.

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1. Introduction

Sweet potato (Ipomoea batatas) is a globally significant food crop, known for its adaptability to diverse agricultural environments and its nutritional value. In Vietnam, sweet potato cultivation is especially prevalent, ranking fourth in national agricultural output following rice, corn, and cassava (GSOV, 2022). The substantial contribution of these crops to the agricultural sector in 2022 underscores their economic and dietary importance in the region. Sweet potatoes are not only consumed directly but are also used in various industries, including feed production, due to their high content of starch, protein, fibers, vitamins, β-carotene, and essential minerals such as iron, calcium, magnesium, and zinc, along with other dietary bioactive compounds (Alam, 2021).

Enhancing the genetic diversity of important food crops such as sweet potatoes is pivotal in modern agriculture. This diversity aims to develop new varieties that can withstand environmental stresses such as drought and salinity, which are increasingly common due to climate change. Additionally, there is a growing demand for varieties with improved nutritional components and higher tuber yields. Mutant technology, which modifies one or more features genetically by applying physical or chemical agents, is a valuable technique in plant breeding (Mohamed et al., 2021). Furthermore, mutagenesis through irradiation combined with in vitro culture techniques is widely employed to introduce beneficial genetic variations (Oladosu et al., 2015).

Gamma rays, known for their deep penetration power, are frequently used in mutation breeding. They can induce significant changes within the cellular structure, affecting redox reactions, metabolic processes, and the synthesis of proteins and hormones, which can lead to cell death or the creation of novel mutations (Roslim et al., 2015). Previous studies have demonstrated the effectiveness of radiation in various ornamental plants such as Saintpaulia, Begonia, Dianthus caryophyllus, Helianthus annuus, and Nicotiana tabacum (Hasbullah et al., 2012). Specifically, research on irradiated sweet potatoes has yielded intriguing results, such as the induction of flesh tuber color mutations observed in plants exposed to gamma rays in field conditions (Wang et al., 2007). Further studies have documented the development of mutant lines with increased salt tolerance following the irradiation of sweet potato tubers (Shaozhen et al., 2009) and variations in tuber yield when stem segments were exposed to ^{60}Co gamma-ray and subsequently regenerated *in vitro* (Shin et al., 2011).

This study aimed to determine the suitable plant growth regulator concentration added to the culture medium for regenerating shoots from shoot tips and nodal segments and to evaluate the effects of varying doses of ^{60}Co gamma-ray irradiation on the survival and mutation formation of these regenerates. The findings will contribute to a foundation for future advances in sweet potato breeding, promoting the combination of irradiation and in vitro culture techniques to enhance stress tolerance, yield, and nutritional quality, thereby contributing to food security and sustainable agriculture in the face of climate change.

2. Materials and Methods

2.1. Plant materials

Young stems of the HL518 sweet potato variety, 15 - 20 cm in length, were sprouted in clean sand. These stems were cut and surface-sterilized using a 20% commercial bleach solution (5% sodium hypochlorite, NaClO) for 15 min to minimize microbial contamination. After sterilization, the nodal segments were excised into 1 cm pieces and cultured on MS medium (Murashige & Skoog, 1962) supplemented with 1 mg/L 6-benzylaminopurine (BAP).

2.2. Shoot induction from shoot tips and nodal segments

Shoot tips and nodal segments measuring 0.5 cm, harvested from 4-week-old sweet potato shoots, were cultured on MS medium augmented with BAP (1.0 and 2.0 mg/L) and indole-3-butyric acid (IBA) at concentrations of 0, 0.2, and 0.5 mg/L to induce shoot formation. The cultures were incubated at a temperature of 25 ± 2°C, under a 16-h photoperiod with 2,000 lux of light provided by fluorescent lamps, and 8 h of darkness. The growth of shoots was recorded after 4 weeks. The optimal concentrations of plant growth regulators (PGRs) determined in this experiment were utilised in subsequent experiments involving shoot regeneration after irradiation.

2.3. Gamma-ray treatment

Shoot tips and nodal segments, precisely 5 mm in length, were positioned on MS medium without plant growth regulators (PGRs). These explants were exposed to ^{60}Co gamma radiation at doses ranging from 0 to 90 Gy and were then immediately transferred to MS medium containing 2 mg/L BAP and 0.2 mg/L IBA. After four weeks, growth indices such as survival, mortality, regeneration percentages, and instances of abnormal growth were meticulously documented. The median lethal dose (LD_{50}), which is the dose at which 50% of the samples are killed, was determined based on the percentage of dead samples (variable y) at different doses of gamma radiation (variable x). Microsoft Excel 2010 was utilized to build a correlation equation between x and y, with LD50 calculated by setting y = 50%.

Each uniquely regenerated shoot line post-irradiation was isolated, dissected into individual nodes, and sub-cultured over four generations to assess mutation induction and recovery from irradiation damage. Growth indicators, including shoot height, petiole length, and leaf length, were recorded after four weeks.

2.4. DNA extraction and SCoT analysis

Leaf samples exhibiting morphological changes from the representative shoot of each observed morphological variant in the first generation were collected for DNA analysis. Genomic DNA was extracted from fresh leaf tissues using the hexadecyltrimethylammonium bromide (CTAB) method (Doyle & Doyle, 1987), with specific modifications to suit our sample type. DNA concentration was quantified using a BioDrop µLite spectrophotometer (Biodrop, England).

PCR amplification was performed with DreamTaq PCR Master Mix (Thermo Fisher Scientific), specific Start Codon Targeted (ScoT) primers at 0.5 µM, 1 µL of template DNA (50 ng/µL), and nuclease-free water up to 12.5 µL under the following conditions: an initial denaturation at 94°C for 60 sec, followed by 40 cycles of 60 sec at 94°C, 60 sec at the specific annealing temperatures for each primer, and 90 sec at 72°C for extension, concluding with a final 7 min of extension at 72°C (Applied BiosystemsTM 2720 Thermal Cycler, USA). The annealing temperatures were as follows: SCoT 1, 20, 21 at 50°C; SCoT 2 at 51°C; and SCoT 5, 11, 14, 15, 35, 36 at 55°C. Amplified products, along with a GeneRuler 1 kb Plus DNA Ladder (Bioline, UK), were resolved on a 1% (w/v) agarose gel (Thermo Scientific, USA) stained with GelRed, and visualized under UV light using the MultiDoc-It Imaging System (UVP) (Fisher Scientific, USA).

ImageJ lab software 2.0 (Bio-Rad, UK) was used to determine the bands appearing on each electrophoresis image. The presence of a band was coded as 1, and the absence as 0, respectively, creating a binary matrix from 10 SCoT primers. The genetic similarity coefficients were analyzed, and a phylogenetic tree was constructed using NTSYSpc 2.1 software (UPGMA method).

2.5. Experimental design and statistical analysis

Completely randomized designs (CRD) were used in all experiments, with each treatment replicated three times. A one-way ANOVA was performed to analyze the data, and Duncan's Multiple Range Test was employed to identify significant differences in means at a 5% probability level. The results are presented as mean ± standard deviation (SD).

3. Results and Discussion

3.1. Effect of BAP and IBA on shoot induction from shoot tip and nodal segment

After four weeks of culture, shoot induction from shoot tips reached 100% on the medium supplemented with 2 mg/L BAP and 0.2 mg/L IBA. Other treatments also showed high percentages of shoot induction, ranging from 80 to 96.7%, suggesting a positive effect of combining BAP with IBA (Table 1). The number of shoots per explant was highest in the medium containing 2 mg/L BAP and 0.5 mg/L IBA, achieving an average of 2.05 shoots per explant, indicating that increased IBA concentration enhances shoot production. The culture medium containing 2 mg/L BAP and 0.2 mg/L IBA produced effective shoot growth, as shown by a shoot height of 4.07 cm and 2.98 nodes per explant (Table 1). The medium with 1 mg/L BAP and 0.2 mg/L IBA resulted in the highest shoot quality (Figure 1).

Table 1. The effect of BAP combined with IBA on the shoot induction from sweet potato shoot tip

BAP (mg/L)	IBA (mg/L)	Shoot induction rate (%)	Number of shoots (shoot/ explant)	Shoot height (cm)	Number of nodes per explant	Shoot quality
1	0	96.7 ± 5.77	1.23 ± 0.50^{b}	3.47 ± 0.26^{ab}	2.29 ± 0.35^{b}	Good
2	0	93.3 ± 11.5	1.34 ± 0.24^{ab}	2.94 ± 0.23^{b}	1.92 ± 0.13^{bc}	Good
1	0.2	93.3 ± 5.77	1.17 ± 0.28^{b}	3.19 ± 0.07^{b}	2.33 ± 0.18^{ab}	Excellent
2	0.2	100 ± 0.00	1.77 ± 0.05a^{b}	4.07 ± 0.38^{a}	2.98 ± 0.26^{a}	Good
1	0.5	90.0 ± 10.0	1.40 ± 0.08a^{b}	3.47 ± 0.23^{ab}	2.34 ± 0.13^{ab}	Good
2	0.5	80.0 ± 10.0	2.05 ± 0.21^{a}	2.98 ± 0.47^{b}	1.37 ± 0.34^{c}	Fair
P		> 0.05	< 0.05	< 0.05	< 0.05	

Means values followed by distinct letters exhibit considerable differences calculated by Duncan's test (Pα = 0.05). BAP: 6-benzylaminopurine; IBA: Indole-3-butyric acid.

Statistical analysis confirmed significant differences among PGR concentrations regarding the number of shoots, shoot height, and number of nodes per explant *(P* < 0.05*)*. However, the percentage of shoot induction did not differ significantly across varying treatments *(P* > 0.05*)*. This indicates that while BAP and IBA concentrations do not affect the percentage of shoot induction, they significantly impact shoot development. The culture medium supplemented with 2 mg/L BAP and 0.2 mg/L IBA was found to be the most suitable for producing a large number of fast-growing, high-quality shoots.

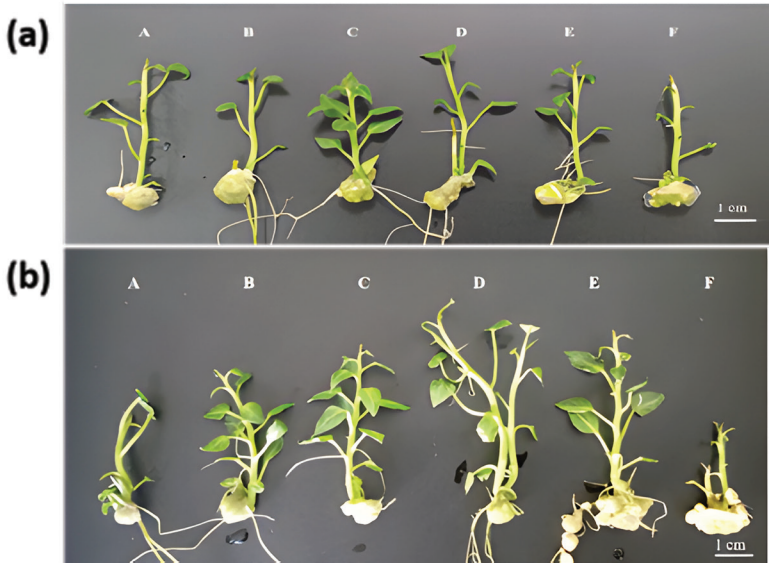


Figure 1. Shoots formed from shoot tip (a) and nodal segment (b) after four weeks cultured on MS medium. (A) 1 mg/L BAP; (B) 2 mg/L BAP; (C) 1 mg/L BAP and 0.2 mg/L IBA; (D) 2 mg/L BAP and 0.2 mg/L IBA; (E) 1 mg/L BAP and 0.5 mg/L IBA; (F) 2 mg/L BAP and 0.5 mg/L. BAP: 6-benzylaminopurine; IBA: Indole-3-butyric acid.

The results showed that shoot regeneration from nodal segments did not vary significantly with different concentrations of BAP and IBA (Table 2). The highest regeneration rate of 100% was achieved in the medium fortified with 1 mg/L BAP. In contrast, when the BAP concentration increased to 2 mg/L, the regeneration rate decreased, ranging from 85.0% to 97.5%, indicating that 1 mg/L BAP is more effective for shoot formation. However, the number of shoots per explant was highest (1.74 shoots) in the medium containing 2 mg/L BAP and 0.5 mg/L IBA, and the greatest shoot height (4.79 cm) was observed with 2 mg/L BAP and 0.2 mg/L IBA. These results suggest that higher BAP concentrations, when combined with lower IBA levels, enhance both shoot quantity and height.

Table 2. Effect of BAP combined with IBA on the shoot induction from nodal segment

BAP (mg/L)	IBA (mg/L)	Shoot induction rate (%)	Number of shoots (shoot/ explant)	Shoot height (cm)	Number of nodes per explant	Shoot quality
1	0	100 ± 0.00	1.00 ± 0.00^{c}	3.91 ± 0.49^{b}	2.78 ± 0.49^{b}	Good
2	0	85.0 ± 19.2	1.00 ± 0.00^{c}	3.73 ± 0.23^{b}	2.74 ± 0.55^{b}	Excellent
1	0.2	92.5 ± 9.57	1.32 ± 0.32^{abc}	4.22 ± 0.16^{ab}	3.11 ± 0.18^{ab}	Excellent
2	0.2	97.5 ± 5.00	1.49 ± 0.24^{ab}	4.79 ± 0.17^{a}	3.74 ± 0.2^{a}	Excellent
1	0.5	100 ± 0.00	1.15 ± 0.13^{bc}	4.31 ± 0.26^{ab}	3.39 ± 0.17^{ab}	Excellent
2	0.5	90.0 ± 8.16	1.74 ± 0.21^{a}	2.48 ± 0.48^{c}	0.92 ± 0.14^{c}	Fair
P		> 0.05	< 0.05	< 0.05	< 0.05	

Means values followed by distinct letters exhibit considerable differences calculated by Duncan's test (Pα = 0.05). BAP: 6-benzylaminopurine; IBA: Indole-3-butyric acid.

Significant variations were also noted in the number of nodes, with the maximum (3.74 nodes per shoot) observed in the medium with 2 mg/L BAP and 0.2 mg/L IBA. This combination also yielded the best overall shoot quality, indicating the effectiveness of BAP and IBA in promoting comprehensive shoot development.

In summary, the combination of 2 mg/L BAP with 0.2 mg/L IBA optimally enhances shoot regeneration, suggesting its utility for *in vitro* sweet potato propagation. The crucial roles of cytokinin BAP in stimulating cell division and auxin IBA in improving shoot quality are supported by the findings of Tu et al. (2019) and Fadaladeen et al. (2022).

3.2. Effects of ^{60}Co gamma-ray irradiation doses on shoot regeneration and morphological variations of sweet potato

Shoot tips and nodal segments were exposed to ^{60}Co gamma rays at doses ranging from 0 to 90 Gy, resulting in rapid chlorophyll loss and mortality within a week. Some of the surviving explants remained alive but did not regenerate shoots, while others produced new shoots with morphological variations (Table 3).

Table 3. Effects of ^{60}Co gamma-ray doses on shoot regeneration and morphological variations of explants four weeks after irradiation

Dose (Gy)	Type and number of explants (n)	Death rate (%)	Shoot induction rate (%)	Morphological variation rate (%)
0	Node (n = 30)	0.0	100	0.0
30	Node (n = 290)	16.2	30.3	2.41
50	Node (n = 290)	39.0	13.1	7.93
70	Node (n = 290)	61.0	15.2	1.38
90	Node (n = 290)	81.4	5.17	0.0
0	Shoot tip (n = 30)	0.0	100	0.0
30	Shoot tip (n = 120)	15.8	66.7	12.5
50	Shoot tip (n = 120)	25.0	68.3	10.8
70	Shoot tip (n = 120)	40.8	26.7	0.0
90	Shoot tip (n = 120)	61.7	17.5	0.0

All percentages were calculated based on the total explant for each treatment (n).

In this experiment, gamma irradiation impacted the explants in three ways: death, survival without regeneration, or limited shoot regeneration. The ratio of each outcome varied depending on the irradiation dose. Increasing radiation dose led to a rise in mortality percentage and a decrease in shoot regeneration rates.

Nodal segments exposed to 30 Gy showed a mortality rate of 16.2% and a regeneration rate of 30.3%, whereas at 90 Gy, the mortality rate rose to 81.4%. Shoot tips showed a similar trend. At 30 Gy, the mortality rate was 15.8%, while the regeneration rate was 66.7%. At 50 Gy, the regeneration rate remained high at 68.3%, but the mortality rate increased to 25%. At 70 Gy, 40.8% of the explants died, and the regeneration rate dropped to 26.7%. The highest mortality rate was 61.7% at 90 Gy. Therefore, LD50 was estimated at 59.3 Gy for nodal segments and 80 Gy for shoot tips (Figure 2).

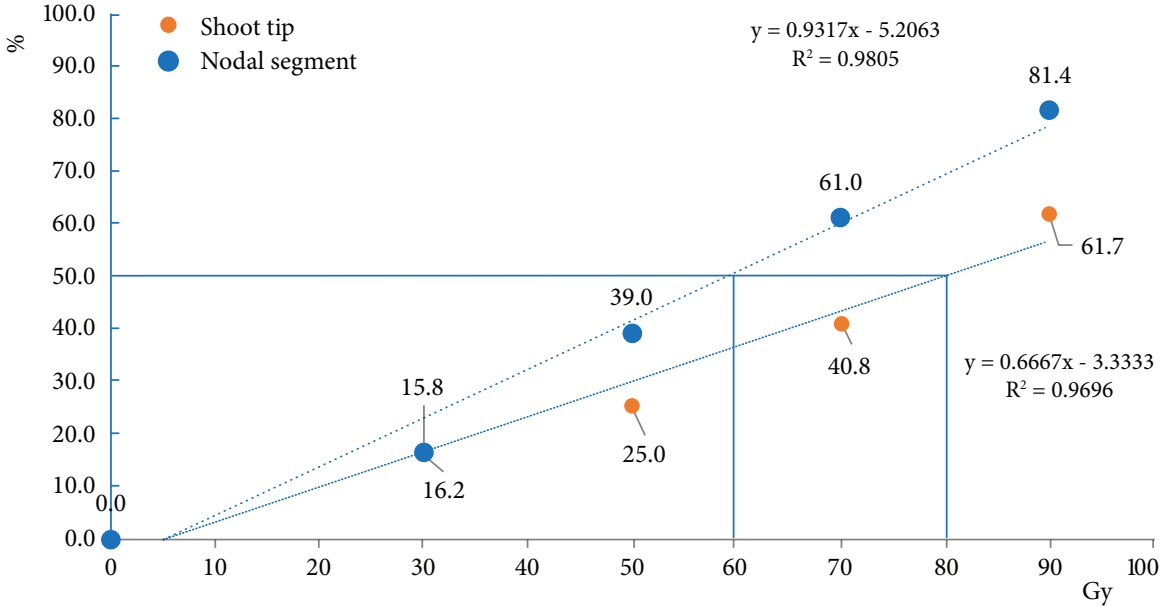


Figure 2. Plot of irradiation doses versus dead explant to calculate LD_{50} of shoot tip and node explants (n-shoot tip = 120; n-node = 290). LD_{50}: Lethal dose 50.

After irradiation, various morphological variations were observed in shoot tips and nodal explants exposed to doses ranging from 30 to 70 Gy (Figure 3). The highest frequency of variations (12.5%) occurred in shoots regenerated from shoot tips exposed to 30 Gy (Table 4). These variations included wavy leaves, split leaves, chlorophyll loss, abnormal root proliferation, and stems without apical meristems. Notably, variations such as wavy leaves, split leaves, and chlorophyll loss were predominantly observed in the first generation (M1), with only one instance of split leaf mutation appearing in the M1V2 generation. At 50 Gy, the mutation frequency was 11.7%, with split leaf mutations being the most common, observed in 12 shoots. No morphological changes were observed at doses of 70 and 90 Gy.

Meanwhile, nodal segments exhibited a greater variety of morphological variations compared to shoot tips. For instance, after irradiation at 30 Gy, 290 nodal segments regenerated 2 shoots with wavy leaves and 5 shoots with split leaves, resulting in a variation frequency of 3.79%. At 50 Gy, the mutation frequency increased to 8.97%, with numerous variants observed including wavy leaves (7), split leaves (7), chlorophyll loss (1), light leaf veins (1), and abnormal root proliferation (10). Abnormal root proliferation, particularly notable for its potential benefits in arid or nutrient-poor soils, was observed only in the first generation after irradiation, characterized by significantly expanded root systems (Figure 4).

The stability of morphological variations varied across generations, with some variations reappearing in later generations. For instance, by the M1V4 generation, explants irradiated at 30 and 50 Gy exhibited the reoccurrence of wavy leaves. Additionally, the M1V4 generation of nodal segments irradiated at 50 Gy showed a new variation, light leaf veins, that was not observed in earlier generations.

In contrast, nodal segments treated with 70 and 90 Gy displayed the lowest variation rates, at 1.38 and 0%, respectively. Explants exposed to 70 Gy exhibited a unique variation of stems without apical meristems (Figure 3h).

Table 4. Number of morphological variants observed across four generations

Shoot tip													
	30 Gy				50 Gy				70 Gy				90 Gy
Variants	M1V1	M1V2	M1V3	M1V4	M1V1	M1V2	M1V3	M1V4	M1V1	M1V2	M1V3	M1V4	(M1V1-M1V4)
Wavy leaf	7	-	-	-	-	1	-	-	-	-	-	-	-
Split leaf	7	-	-	-	13	-	-	-	-	-	-	-	-
Chlorophyll loss	1	-	-	-	-	-	-	-	-	-	-	-	-
Frequency of variation (%)	12.5				11.7				0				0
Number of variation types observed	3				2				0				0
Nodal segment													
	30 Gy				50 Gy				70 Gy				90 Gy
Variants	M1V1	M1V2	M1V3	M1V4	M1V1	M1V2	M1V3	M1V4	M1V1	M1V2	M1V3	M1V4	(M1V1-M1V4)
Wavy leaf	2	2	-	2	2	3	-	2	-	-	-	-	-
Split leaf	5	-	-	-	6	1	-	-	3	-	-	-	-
Chlorophyll loss	0	-	-	-	1	-	-	-	-	-	-	-	-
Abnormal root proliferation	-	-	-	-	10	-	-	-	-	-	-	-	-
Light leaf veins	-	-	-	-	-	-	-	1	-	-	-	-	-
Stem without apical meristem	-	-	-	-	-	-	-	-	1	-	-	-	-
Frequency of variation (%)	3.79				8.97				1.38				0
Number of variation types	2				5				1				0



Figure 3. Morphological variations produced by ^{60}Co gamma irradiation in sweet potato. (a, b) split leaves; (c) chlorophyll loss; (d) light leaf vein; (e, f) wavy leaves; (g) non-irradiated shoot; (h) stem without apical meristem; (1 - 3) split leaves; (4) chlorophyll loss; (5-7) wavy leaves. Scale bar = 1 cm.



Figure 4. Abnormal root proliferation after irradiation with ^{60}Co gamma-ray at 50 Gy (a) and root in control treatment (b).

The mutation frequency increased from 3.79% at 30 Gy to 8.97% at 50 Gy, indicating that higher radiation doses cause more extensive cellular damage and induce greater morphological changes. This result aligns with previous studies in *Curcuma*, where similar effects were observed (Chusreeaeom et al., 2019). Morphological variations observed include split leaf apices and wrinkled leaves in *Curcuma heyneana* (Hapsari et al., 2021), elongated stems lacking apical meristems (Lage et al., 1992), altered flower colors in roses (Bala et al., 2015), and disrupted chlorophyll synthesis (Hapsari et al., 2021). These changes likely stem from radiation-induced genetic alterations such as point mutations, insertions, deletions, or duplications, which can lead to abnormal phenotypes, growth patterns, reproductive behaviors, or stress responses (Mohamed et al., 2021; Puripunyavanich et al., 2022).

Gamma rays disrupt essential metabolic processes including protein biosynthesis, hormone balance, and enzyme function, which may result in cell death or mutations (Katiyar et al., 2022). High radiation doses exacerbate oxidative stress and adversely impact plant growth and development (Choi et al., 2021). Different types of plant explants, such as callus, petioles, tubers, and shoots, exhibit varying degrees of susceptibility to radiation injury (Hasbullah et al., 2012; Bado et al., 2016). For instance, the LD50 values vary among tissues, such as potato tuber (7.3 - 13 Gy) and new shoot sprouts (20.6 - 54.8 Gy) (Bado et al., 2016).

3.3. Restoration of putative mutants across sub-culture cycles

To assess the response of irradiated explants over successive sub-culture cycles, we monitored their recovery trend over four generations (M1V4, Figure 5). The data indicate that growth indicators approached those of control explants by M1V4. Recovery varied between shoots regenerated from irradiated nodes and shoot tips. Nodal segments irradiated at 50 Gy showed significant recovery, with shoot height increasing from 1.16 cm in the first generation to 4.61 cm in the third generation. Similarly, petiole length increased from 1.13 to 1.9 cm, and leaf length from 1.04 to 1.53 cm. Shoot tips exhibited comparable recovery capabilities, with shoot heights increasing to 3.76 (30 Gy) and 3.70 cm (50 Gy) by the third generation, respectively. Across all treatments, growth indicators stabilized after the fourth generation. However, explants exposed to 70 and 90 Gy did not show significant recovery. Regenerated shoots from explants exposed to 30 or 50 Gy displayed greater leaf and petiole lengths compared to controls, suggesting radiation-induced growth stimulation. This contrasts with the growth inhibition observed at higher radiation doses (Figure 5).

The observed effect aligns with previous findings by Qi et al. (2015) and Hapsari et al. (2021), suggesting that irradiation enhances cellular processes such as division and enzyme synthesis, potentially increasing endogenous growth regulator production or cellular dimensions (Lage et al., 1992; Ramchander et al., 2024; Wahyudi et al., 2024). Gamma-ray irradiation temporarily halts mitosis, explaining the initially low growth indices in the first generation, which subsequently improve by the third (Canti & Spear, 1929). However, higher radiation doses diminish growth and recovery potential. Over time, shoots recover and enhance attributes such as shoot height, leaf length, and petiole length, likely due to cellular repair mechanisms, as observed in rice (*Oryza sativa* L.) (Kariuki et al., 2019) and banana (*Musa acuminata*) (Hasim et al., 2021).

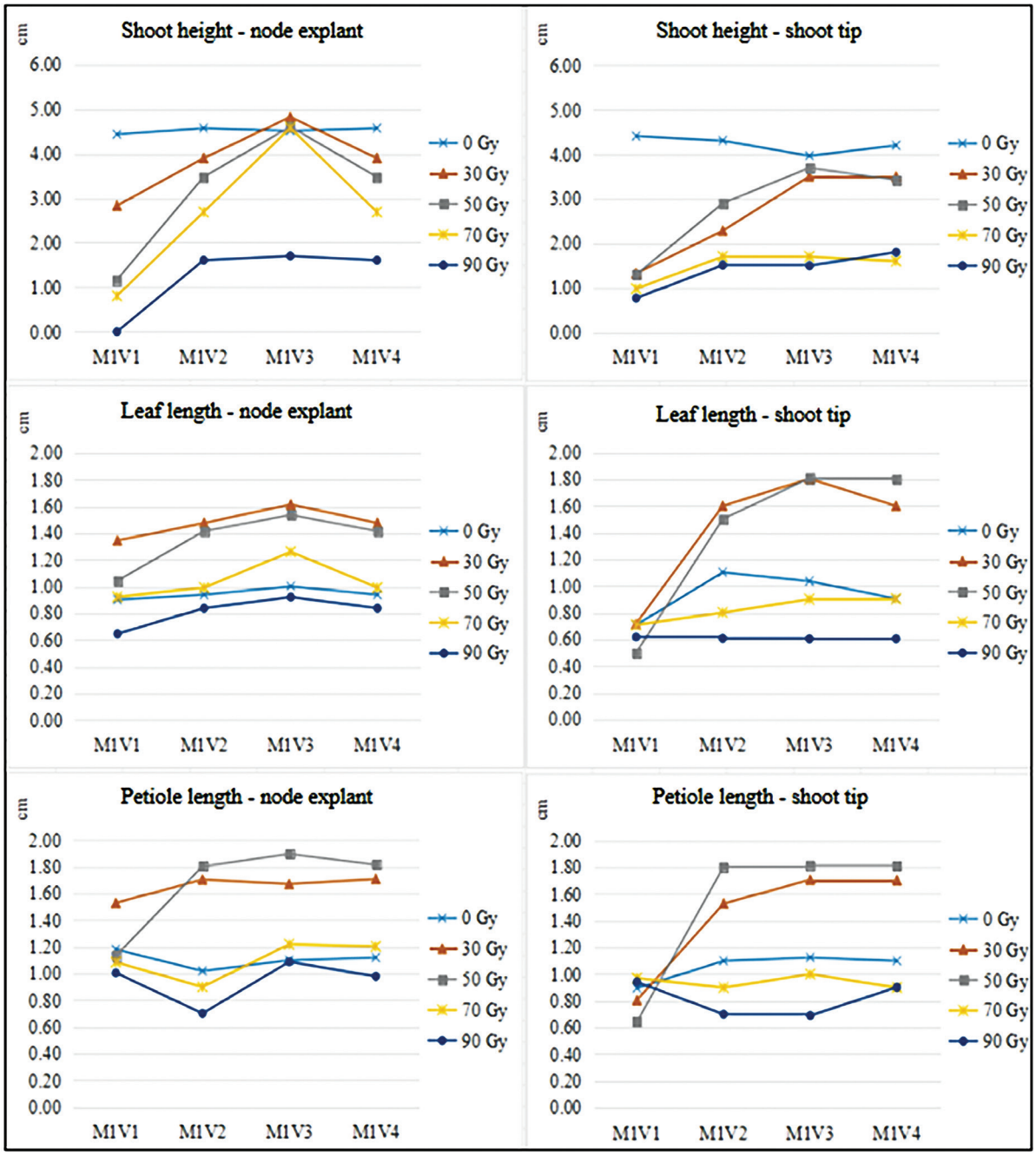


Figure 5. Growth indicators of shoot regenerating across four generations.

3.4. Identification of genetic variants using SCoT markers

SCoT markers are designed to identify genetic variations by targeting start codons within small gene regions capable of encoding proteins (Collard & Mackill, 2009). This method has shown high potential in assessing genetic diversity in sweet potatoes (Aswathy et al., 2016). Using ten primers, the analysis detected a total of 93 bands, averaging 9.3 bands per primer with variability ranging from 4 to 16 bands per primer. The polymorphism ratio ranged from 0 to 42.9%, averaging 23.8%. Particularly noteworthy was SCoT 2 primer, which exhibited the highest polymorphic band percentage at 42.9%, where 3 out of 7 bands were polymorphic (Table 5).

Table 5. Primer sequence, number of amplified products, and percentage of polymorphism

Primer name	Primer sequence (5′-3′)*	No. of amplified bands	No. of polymorphic bands	Percentage of polymorphic bands
SCoT 1	CAACAATGGCTACCACCA	5	0	0.0
SCoT 2	CAACAATGGCTACCACCC	7	3	42.9
SCoT 5	CAACAATGGCTACCACGA	5	1	20.0
SCoT 11	AAGCAATGGCTACCACCA	3	1	33.3
SCoT 14	ACGACATGGCGACCACGC	12	3	25.0
SCoT 15	ACGACATGGCGACCGCGA	12	3	25.0
SCoT 20	ACCATGGCTACCACCGCG	16	3	18.8
SCoT 21	ACGACATGGCGACCCACA	14	3	21.4
SCoT 35	CATGGCTACCACCGGCCC	15	4	26.7
SCoT 36	GCAACAATGGCTACCACC	4	1	25.0
Sum		93	22	
Mean		9.3	2.2	23.8

*Note: *: Aswathy et al. (2016); ScoT: Start Codon Targeted.*

Genetic similarity indices among the tested sweet potato samples ranged from 0.419 to 0.957, with an average value of 0.734. The highest similarity coefficient (0.957) was observed between the mother plant samples and non-irradiated explants (control), indicating minimal genetic variation attributable to subculturing alone. Conversely, the lowest similarity coefficient (0.42) was found between Thai varieties and i*n vitro* plantlets, suggesting significant genetic differentiation (Table 6).

Table 6. Genetic similarity between mother and putative mutants of sweet potato

	MOT	IVP	S30	S50	WAV	SPL	LVL	ISR	THA	PFV	WFV
MOT	-										
IVP	0.957	-									
S30	0.699	0.720	-								
S50	0.785	0.806	0.849	-							
WAV	0.677	0.677	0.677	0.698	-						
SPL	0.656	0.677	0.720	0.720	0.677	-					
LVL	0.677	0.699	0.763	0.742	0.720	0.677	-				
ISR	0.527	0.527	0.634	0.570	0.570	0.591	0.656	-			
THA	0.441	0.419	0.484	0.484	0.484	0.462	0.484	0.613	-		
PFV	0.559	0.581	0.688	0.645	0.559	0.581	0.645	0.624	0.495	-	
WFV	0.548	0.570	0.656	0.613	0.548	0.548	0.634	0.613	0.484	0.946	-

Note: MOT: Mother plants; IVP: In vitro plants; S30: Stimulate at 30 Gy; S50: Stimulate at 50 Gy; WAV: Wavy leaf; SPL: Split leaf; LVL: Light Leaf Vein; ISR: Israel variety; THA: Thailand variety; PFV: Purple fleshed variety; WFV: White fleshed variety.

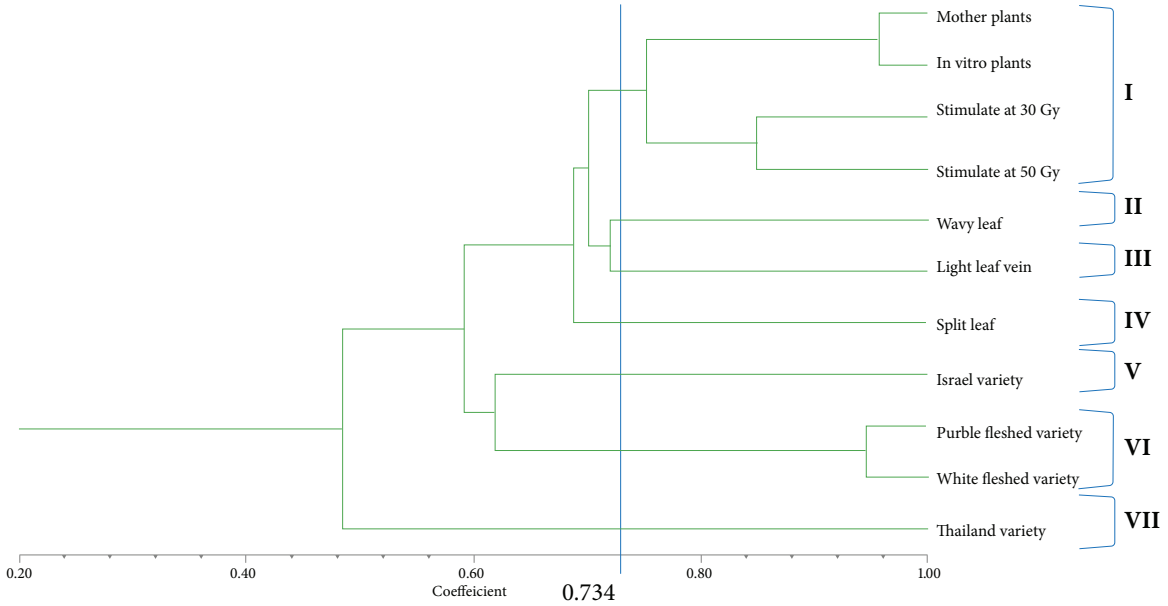


Figure 6. Genetic variation in putative mutants analysed using ten SCoT markers with the UPGMA method.

The genetic clustering diagram, with an average genetic similarity coefficient of 0.734, categorizes 11 samples into seven distinct groups (Figure 6). Group I includes the mother plant, *in vitro* plants, and those stimulated by 30 and 50 Gy irradiation. Groups II, III, IV, and V correspond to variants with wavy leaves, light leaf veins, split leaves, and the Israeli variety, respectively. Group VI encompasses purple and white-fleshed varieties from Vietnam, while Group VII comprises the Thai variety.

Each leaf morphological variant exhibits genetic distinctiveness from one another as well as from both the in vitro control plants and the mother plant, with genetic similarities ranging from 0.656 to 0.763. Explants stimulated at 30 or 50 Gy show minor genetic differences between them (0.849). This study underscores the effectiveness of 10 SCoT markers in assessing genetic relationships among irradiated and non-irradiated sweet potato samples, demonstrating their utility in detecting polymorphic bands and distinguishing sweet potato lines (Aswathy et al., 2016). Additionally, SCoT markers have successfully evaluated genetic diversity in other species such as rice (Collard & Mackill, 2009), *Cocos nucifera* L. (Rajesh et al., 2015), and Abiu germplasm (Peng et al., 2023). Gamma-ray radiation has also been shown to induce genetic changes in shoot lines, creating genotypes that differ from the parent plant, as previously observed in Lilium (Aslam et al., 2016), *Gerbera jamesonii* (Hasbullah et al., 2012), *Celosia argentea* (Kurucz et al., 2022) and *Pogostemon cablin* Benth (Normasari et al., 2023).

4. Conclusions

This study successfully identified MS medium supplemented with 2 mg/L BAP and 0.2 mg/L IBA as optimal for promoting shoot growth and regeneration in sweet potatoes. The research underscores the significant impact of ^{60}Co gamma irradiation on *in vitro* sweet potato vitality. Exposure of shoot tips and nodal segments to gamma rays resulted in notable mortality and a range of morphological variants, including wavy leaves, split leaves, chlorophyll loss, abnormal root proliferation, and stems lacking apical meristems. Importantly, all observed leaf variants, such as split leaf, light leaf vein, and wavy leaf, were confirmed to be genetically distinct from the original plant using SCoT markers. This study contributes to our understanding of the mutations induced by irradiation in sweet potatoes, thereby advancing knowledge on the biological effects of gamma ray radiation on this crucial food crop. Furthermore, these findings lay a foundation for future research in genetic enhancement and breeding programs.

Conflict of interest

The authors declare that they have no competing interests.

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