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Morphogenesis, Estimated Allele Frequency, and Heterozygosity in The Putative Mutation Population of *Solomon* Teak (M1V1)

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ABSTRACT
In vitro continuous development (proliferation) and growth enhancement of callus, which orginated from irradiated-plantlets of *Solomon* teak clones, have been sequentially conducted on culture medium of (1) MS + 0.1 ppm kinetin, then transferred to (2) an half-strength MS + 0.1 ppm kinetin + 0.1 ppm BAP + 100 ppm charcoal, to (3) an half-strength MS + 0.1 ppm BAP + 3% sugar and finally to (4) half strength MS + 0.3 ppm BAP + 3% sugar. The objectives of this research were to evaluate the morphogenesis of putative-mutant *Solomon* teak from generation M1V1 and estimate allele frequencies as well as population heterozygosity in vitro. Results demonstrated that exposure to gamma irradiation (10, 20, 30, and 40 Gy) in the M1V1 generation induced callus formation within two weeks, followed by the development of embryogenic callus. Genetic analysis revealed low diversity among M1V1 individuals as indicated by Na>Ne and He<Uhe. Gamma irradiation potentially increased both allele number and heterozygosity by approximately 50%. Ten putative-mutant teak seedlings are obtained, these lines should be maintained and propagated as valuable genetic resource for future teak improvement programs.

Keyword: Callus, Embryogenic, *In vitro*, Kinetin, Proliferation

1. Introduction

Teak is a deciduous plant with both complete (hermaphroditic) and compound flowers. It exhibits an open pollination system (chasmogamy) and is predominantly self-incompatible (96-100%), which promotes cross-pollination and enhances fruit set [1]. The pollination is primarily facilitated by insects such as *Braunsapis sp.* (*Apidae*), *Ceratina sp*. (*Apidae*), and *Nomia sp*. (*Halictidae*) [2]. Additionally, wind and water act as secondary pollination agents, capable of dispersing pollen over distances of over 350 m in natural forests [3]. Pollen flow contributes directly to gene flow, which in turn influences genetic variation within populations [4]. Genetic variation manifests in both quantitative and qualitative traits, controlled by multiple genes (polygenic traits) or a single gene (pleiotropy). Key traits targeted for improvement of teak productivity include reducing harvesting time and enhanced growth in height and diameter. However, limited quantitative trait variation is associated with overall low genetic variation in teak population. This low genetic variation [5] is due to the scarcity of wild and monophyletic germplasm sources [6]. Increasing teak genetic diversity can be accomplished by increasing gene flow [7], which plays a critical role in development of desirable quantitative traits.

Hybridization and tissue culture techniques have also been employed to support the enhancement of these traits in teak [8].

Tissue culture has been widely used to conserve rare species, such as *Shorea stenoptera* Burck [9], and to obtain superior (high productive) clones, as demonstrated in teak (*Tectona grandis*) [10]. Superior clones can be created by conserving and propagating elite genotypes, while somaclonal variation in inferior traits through *in vitro* culture [11]. The induction somaclonal variation can result in the expression of favorable traits in otherwise inferior plants, increasing their potential as superior clones 12]. For instance, the addition of adenine sulfate has been shown to increase genetic variation by approximately 3% [13]. However, the desired quantitative traits resulting from somaclonal variation are often classified as temporary or putative mutations, which are typically not heritable. Several factors contribute to these transient genetic changes, including high concentrations of inorganic nutrient content in the culture medium [14], repeated sub-culturing, exposure mutagenic agents, and specific culture conditions [15]. To transform these temporary mutations into stable, heritable (definitive) mutations, tissue culture can be combined with mutation induction techniques. The integration of *in vitro* culture methods with gamma irradiation has proven effective in rapidly enhancing genetic diversity [16].

The effectiveness and efficiency of gamma-ray irradiation play a critical role in determining mutation frequency and occurrence of chimeras [17]. These outcomes are largely influenced by irradiation dose, as well as by adverse biological responses such as chromosomal aberrations, plant mortality, and reduction in chlorophyll content [18]. In previous research, gamma irradiation resulted in reductions in chlorophyll content in *Rosa multiflora* [19] and induced chromosomal mutations in *Arabidopsis thaliana* [20]. These effects are intimately tied to key biological determinants, including genome size and number, cellular characteristics, physiological responses, development stage, morphological traits, and the post-irradiation environment [21]. Teak's biological response has been estimated using the logarithmic Gaussian model, which determined the LD_{50} (lethal dose for 50% of sample) at 24.5 Gy and RD_{50} (reduction dose for 50 % growth inhibition) at 7.8 Gy [5]. These dosages are critical influencing the frequency of cumulative mutation alleles responsible for the expression of superior traits. Such traits affect both plant morphology (phenotype) and genetic composition. The dominance of genetic material in *Solomon* teak can be attributed to *in vitro* mutagenesis and genetic factors, which have the potential to be used as early selection markers [5]. Early selection can be conducted *in vitro* by observing morphological changes and genetic composition across among gamma-ray treatments [22]. *In vitro*, morphological alteration can be observed based on ontogenetic structural changes in putative mutation plants, from callus to the formation of bipolar organs. Furthermore, plant polymorphism influence morphogenesis pathways associated with potential mutations in *Solomon* teak plants. Polymorphism determines the genetic variation particularly in relation to heterozygosity and allele frequency among putative-mutants [23].

Molecular markers are requirement for estimating genetic differences and relationships among putative-mutant of *Solomon* teak in the M1V1 generation. There have been documented efforts to use molecular markers to assess genetic diversity and relatedness in various plant species. DNA markers technique based on PCR, particularly Random Amplified Polymorphic DNA (RAPD), are commonly used for this purpose [24]. Random Amplified Polymorphic DNA (RAPD) is a dominant marker that is widely used for genetic mapping, mutant analysis, and somaclonal variation *in vitro* culture. It amplifies random segments of genome, without the need for specific primers, allowing broad genome coverage [25]. The use of molecular markers significantly enhances the accuracy of mutant diversity analysis results, for selection purposes in identifying superior clones [26]. The purpose of this study is to evaluate the morphogenesis of putative-mutant *Solomon* teak from generation M1V1 and estimate allele frequencies under *in vitro* conditions

2. Materials and Methods

2.1. Teak Morphogenesis

Mutation induction was carried out using a Gamma Chamber Cobalt-60 (^{60}Co) irradiator at the Laboratory of the Center for Isotopes and Radiation Application Technology, National Research and Innovation Agency. The plantlets originated from shoot cultures collected from the laboratory of Research Center for Biological Resources and Biotechnology, IPB University. Following After irradiation, plantlet was conducted at the Tissue Culture Laboratory of the Department of Forest Resources Conservation and Ecotourism, IPB University. Each experimental unit consisted of 5 plantlets with 4 replications at 5 levels of gamma radiation

dosage (0, 10, 20, 30, and 40 Gy), yielding 100 plantlets on MS0 media, denoted as M1V0 (Figure 1). After one month of irradiation, these plantlets were multiplied using MS + 0.1 ppm kinetin media, and the result is known as M1V1. This procedure was conducted to prevent diplontic selection. Subsequently, all callus derived from M1V1 individuals were multiplied and proliferated on various culture media. The growth and proliferation of the callus, along with the acclimatization process of individual shoots regenerated from M1V1 callus on different media, are presented in Table 1.

Generation M1V1 was also observed for growth and development, as well as factors that may disrupt its growth and development caused by biotic pathogens (fungi, bacteria, and so on) and disturbances caused by its development, both of which experience physiological disorders. The collected data was analyzed and documented using photography to visualize generation development.

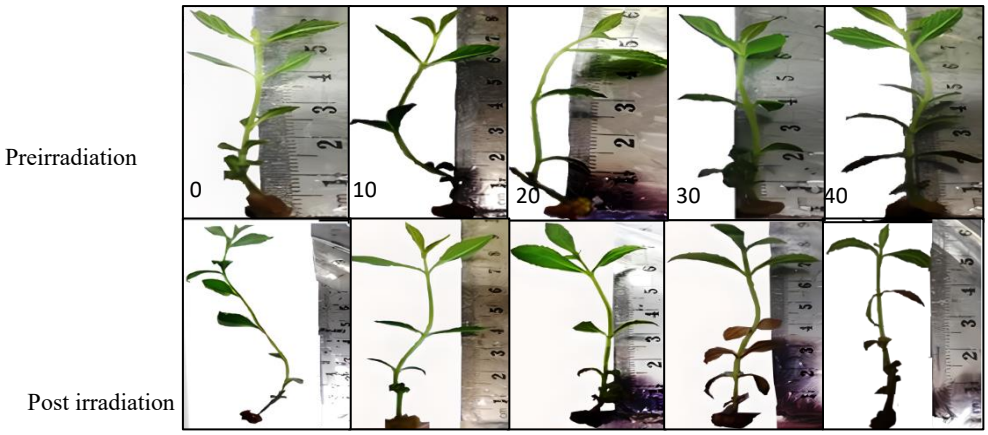

Figure 1. The response timeframe after irradiation

2.2. Materials and DNA Extraction

The DNA extraction was carried out at laboratory of forest genetics and molecular forestry, IPB university Extracting DNA was used method of CTAB (*Cetyl Trimethyl Ammonium Bromide*) with some modification [27]. Each gamma irradiation treatment involved three extractions of approximately ± 5 grams of M1V1 callus. After extraction, the DNA was electrophoresed on a 1% agarose gel. DNA samples (2µL) were combined with a 3µL solution of 10x blue juice. The DNA electrophoresis results were photographed under UV light and analyzed. The RAPD amplification method used OPC primers such as OPC1, OPC2, OPC3, OPC8, OPC9, and OPC10 [5]. The PCR amplification results were electrophoresed on a 1% agarose gel with 33 µL of TEA buffer and 1 µL of gel red for 20-30 minutes. The electrophoresis results were then photographed under UV light for analysis and interpretation (Figure 2).

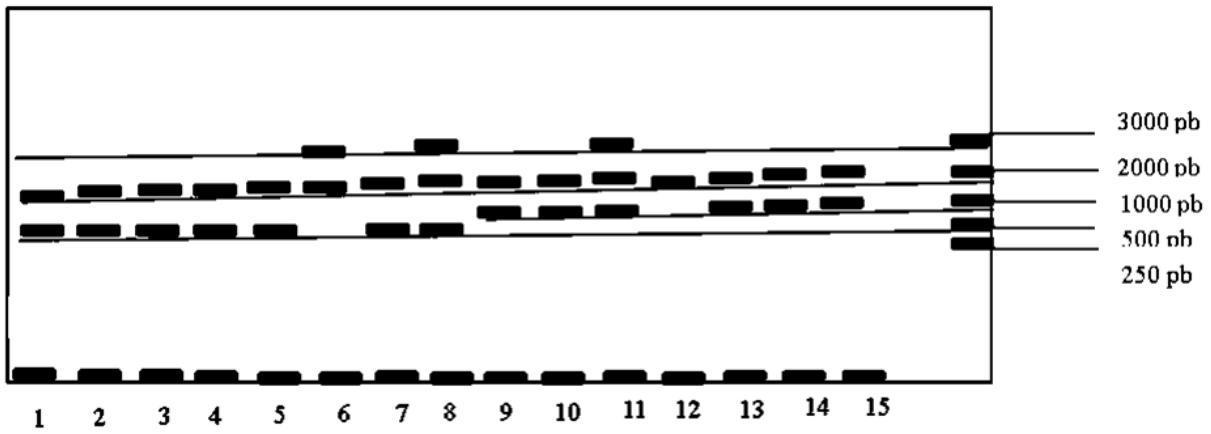

Figure 2. Profiling PCR results that have been amplified using OPC 8 primers. 1 = Control I; 2 = Control II; 3 = Control III; 4 = 10 Gy I; 5 = 10 Gy II; 6 = 10 Gy II; 7 = 20 Gy I; 8 = 20 Gy II; 9 = 20 Gy III; 10 = 30 Gy I; 11 = 30 Gy II; 12 = 30 Gy III; 13 = 40 Gy I; 14 = 40 Gy II; 15 = 40 Gy III

2.3. Analysis of allele frequency estimation

PCR amplification result, document as gel images, were scores using binary values (0 for ansence and 1 for presence of bands). Nei's Genetic Diversity (H) [28] and Nei Genetic Distance (D) [29] were determined using the popGene 1.32 program [30]. GenAlEx 6.0 [31] was used to estimate allele frequencies and analyze heterozygosity.

Formula for determining allele frequency
He = 2 × pq
The value of q is obtained by the formula q = (1 − frequency of the recessive allele)0.5 and $p = 1 - q$

$$UHe = \left\{\frac{2n}{2n-1}\right\} X\ He \quad (1)$$

$$Ne = \frac{1}{p^2 + q^2} \quad (2)$$

Explanation:
He : Expected heterozygous allele
uHe : Unbiased Expected Heterozygosity
Na : Actual allele count
Ne : Effective allele count
P : Frequency of homozygous dominant allele
Q : Frequency of homozygous recessive allele

2.4 Acclimatization of putative-mutant Solomon shoots post-irradiation

The all shoots regenerated from M1V1 callus were ready for greenhouse acclimatization at 35 weeks at the Silviculture Tropica facility, IPB University. The shoots were detached from the callus and acclimatized to form seedlings on a sand, compost, charcoal and husk medium (1:1:2 V/V/V). These observations included plantlets that can become seeds, root development, and the growth of suspected *Solomon* teak mutants. The collected data was examined and documented using photography to visualize the evolution of each generation.

3. Results and Discussion

After irradiation, the plantlets are grown in MS0 medium to neutralize the media and prevent endogenous hormones from influencing them. According to the findings of this study, in treatment 30 and 40 Gy M1V0, the leaves began to become yellow in the first week, beginning with the veins. By the third week, several of the plantlet leaves have dried and lost owing to gamma radiation. After one month of irradiation, generation M1V1 is observed for morphogenic.

3.1 Solomon Teak Morphogenesis

Plant morphogenesis is a complex basic biological phenomenon [32] that is influenced by endogenous variables via phytohormones that can affect plant growth and development [33]. These hormones are involved in cell division, growth, and differentiation processes, as well as their combination in morphogenesis [34]. The involvement of these hormones significantly influences the rate of plant morphogenesis and regeneration capacity, with each plant species exhibiting a distinct morphogenetic response. Exogenous light factors (photomorphogenesis) and growth regulator factors (morphoregulatory) can stimulate faster morphogenesis.

This study demonstrates that morphogenesis involves an increase in callus formation in M1V1 on MS + kinetin media. Gamma-rays can reduce and hinder plantlet regeneration while increasing callus formation, necessitating effective dosages and efficiency to induce plantlets and multiply *Solomon* teak plantlets. The morphological response form following irradiation also indicates the effectiveness and efficiency of the treatments. Callus morphogenesis was initiated on Murashige–Skoog medium supplemented with 0.1 ppm kinetin and exposed to radiation dosage of 10, 20, 30, and 40 Gy. After two weeks, callus formation was evident, and by the third week, the tissues had differentiated into embryogenic callus exhibiting a greenish yellow hue. In *Coffea arabica* callus, gamma-rays are applied embryogenic [35].

The response on teak plantlets in producing callus shows that gamma-rays stimulate endogenous components, primarily by auxin and cytokinin, as similary observed in *Catharanthus roseus* [36] and *Sesamum indicum* [37]. Furthermore, callus obtains from teak plantlet subcultures exhibites friable callus with a brown tint, whereas callus obtained from 20, 30, and 40 Gy dosages produced compact, green, and yellowish callus. Friable callus can be propagated through suspension culture and protoplast fusion, but compact callus, which is thought to be a somatic embryogenic callus, can create new regenerative plantlets.

The volume of the callus represents the activity and number of proliferating tissue cells [38]. Divided tissue illustrates the growth and development phases of callus, which consist of five phases: (1) Lag phase (division preparation). (2) The exponential phase (maximum division). (3) linear phase (phase of maximal reduction and rise in volume). (4) Decline phase (highest reduction in cell division). (5) stationary phase, or continuous growth and cell number [39]. Table 1. describes the growth, development, and regeneration stages of teak plantlet callus after irradiation to acclimatization in grasshouse.

Table 1. The morphogenesis phases observed during proliferation of teak plantlet callus following gamma irradiation

No	Growth and development phases	Visualization of images	Description
1	*Lag phase*	1.1  Explant segment	Initial initiation
		1. 2  Cell division has occurred	Embryogenic callus after 4 weeks in MS + 0.1 ppm kinetin medium
2	*Exponential phase*	 Callus growth rate has maximized	Callus is 14 weeks old
3	*Linear phase*	 Callus size is increased	18-week-old callus with ½ MS + 0.1 ppm BAP media + 0.1 ppm kinetin + 100 ppm charcoal
4	*Decline phase*	 The size of callus is maximum	A 21-week-old callus formed on ½ MS 0.3 ppm BAP 3% sugar.
5	*Stationary phase*	5.1  Callus initiated the shoot.	Callus formed in ½ MS 0.1 BAP with 3% sugar At 28 weeks
		5.2  Bipolar callus.	Callus regenerated at 35 weeks in ½ MS 0.1 BAP + 3% sugar.
		5.3  Seedling	Seedlings at 4 months with sand: Compost, charcoal, and husk (1:1:2 V/V/V).

Table 2. Causes of growth abnormalities in M1V0 and M1V1 plantlets at 16 weeks after culture (WAC)

Dosage (Gy)	Types of disorder causes			
	Bacteri	Fungi	Hyperhydricity	Albinism
M1V0				
0	0	5/20	0	0
10	2/20	3/20	0	0
20	0	1/20	1/20	1/20
30	0	1/20	0	2/20
40	0		0	2/20
M1V1				
0	2/71	17/71	0	0
10	2/63	6/63	0	1/63
20	3/58	7/58	0	0
30	0	4/50	0	0
40	0	3/32	0	0

M1V0: Post-irradiation plantlets using MS_0 medium; M1V1: Subculture with MS + 0.1 ppm kinetin

3.2. Causes of Growth Disorders in Plantlet Generations M1V0 and M1V1

The contamination causes physiological abnormalities, that can result in planlet death. Plantlets, fungi, and bacteria are competing for the limited nutrients in the culture medium. Hyperhydricity and albinism can also interfere with the formation and development of M1V0 and M1V1 generations (Table 2). Hyperhydricity can be caused by an increase in ROS (*Reactive Oxygen Species*) in the form of phenolic compounds [40], nutritional element inhibition, and gas exchange through the active route due to the water-filled apoplast [41]. Albinism is caused by a lack of chlorophyll pigments, which disrupts both anabolic and catabolic metabolism. Those symptoms can be shown in Figure 3.

Gamma irradiation can cause physiological and non-physiological disordes. Plantlet mortality can be caused by both physiological (fungi and bacteria) and non-physiological (albino and hyperhydric conditions). Physiological disorders are frequently attacked by *Aspergillus sp*. and *Penicillium spp*. till they perish. Penicillium sp. appear whitish-yellowish (Figure 3). Mutations caused by gamma-ray irradiation can also result in albino plantlets.

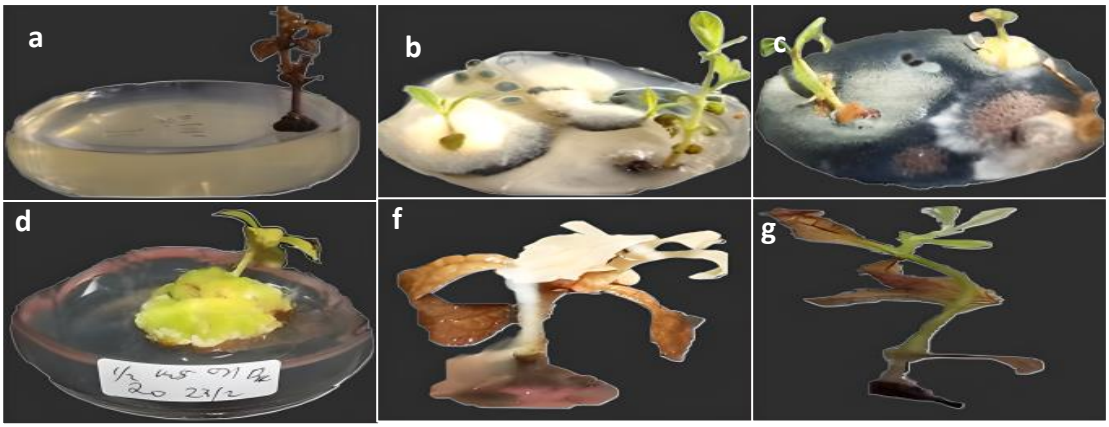

Figure 3. Plantlet growth abnormalities can be caused by: a) permanent death; b) *Penicillium* sp. fungi; c) *Aspergillus* sp. fungi; d) bacteria; e) hyperhydricity; and f) albinism.

3.3. Callus Formation Response on MS Medium with 0.1 ppm Kinetin (M1V1)

In the second week, all individual M1V1 treatments and the control cause swelling in on MS + 0.1 ppm kinetin media. Swelling begin at the bottom stem of plantlets in contact with the media. At 4 weeks old, the plantlet leaves twisted and wrinkled (*rosette*), followed by senescence and an increase in callus volume (Figure 4). This suggests that the addition of kinetin can promote callus development.

Gamma-ray irradiation (10-40 Gy) stimulates callus growth, both embryogenic and non-embryogenic. The 10 Gy treatment produces a non-embryogenic callus (Figure 4), but the 20, 30, and 40 Gy treatments produce an embryogenic callus. Color and structure can help distinguish between embryogenic and non-embryogenic calluses. Non-embryogenic calluses are white and friable in structure, whereas embryogenic calluses are often yellow and compact [42]. The number of embryogenic calluses on MS + 0.1 ppm kinetin M1V1 medium is shown in Table 4. Furthermore, at levels below 20 Gy (10 ≤ gamma-ray irradiation dose <20 Gy) and below 40 Gy (20 ≤ gamma-ray irradiation dose ≤40 Gy), are different callus shapes and colors. Doses less than 10 Gy generally create brown-colored and friable callus, but doses greater than 20 Gy (20, 30, and 40 Gy) produce compact and greenish-yellow callus. Callus produced by 10 Gy often creates phenolates at a faster rate than callus produced by doses more than 20 Gy, causing the callus to turn brown faster (Figure 4).

Green calluses suggest vigorous cell proliferation, whereas dark calluses indicate phenolic component oxidation. Non-embryogenic callus is more prone to browning than embryogenic callus because it produces phenolic chemicals at a rate proportional to callus expansion [43]. The number of embryogenic callus on MS + 0.1 ppm kinetin media can be seen in Table 3.

Table 3. M1V1 growth in MS+0.1 ppm kinetin medium.

Radiation dosage (Gy)	The quantity of explants obtained	Growth response		Description
		Callus Formation	Shoot Growth	
0	71	8	63	Shoots grow normally
10	50	49	1	Non-embryogenic callus growth is more dominating
20	58	56	2	Callus growth is more dominating
30	50	50	0	Callus growth is more dominating
40	32	32	0	Callus growth is more dominating.

3.4. Heterozygosity of Post-Irradiation Solomon Teak (M1V1)

Mutations within the *Solomon* teak population, indicated by deviations from Hardy–Weinberg equilibrium, can be assessed through measures of allelic diversity (Na and Ne) and population diversity, including Shannon's information index (I) and the percentage of polymorphism. Different allele diversity values (*Na≠Ne*) suggest uneven allele frequencies at each locus [44]. A Na value less than Ne indicates low allele frequencies at each locus in the population, whereas a Na value greater than Ne suggests high allele frequencies at each locus. The proportion of homozygous (*dominant and recessive*) and heterozygous alleles within each population of gamma-ray irradiation dose treatments is used to express allele frequency. Heterozygotes are DNA that undergo mutation due to gamma radiation, and heterozygotes in many species and populations are called polymorphisms. Polymorphism and Shannon's information index (I) can be used to calculate population diversity at different gamma-ray irradiation doses. Shannon's information index is also used to determine population variance and variety [45]. Table 4 displays the values for Na, Ne, I, He, Uhe, and percentage polymorphism.

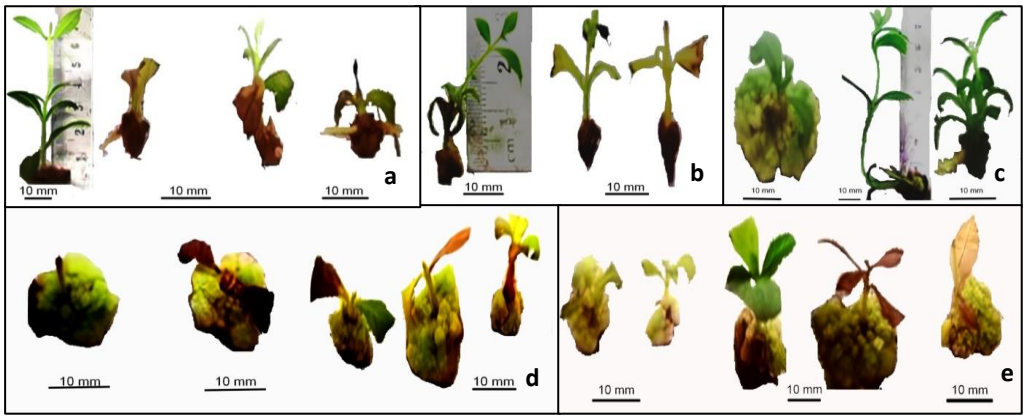

Figure 4. Callogenesis was observed in all gamma-ray irradiation treatments **a** (0 Gy), **b** (10 Gy), **c** (20 Gy), **d** (30Gy), and **e** (40 Gy) in MS+0.1 ppm kinetin medium

Table 4. The estimated allele frequency and heterozygosity within the population of gamma-ray irradiation dosages**.**

Dosis	N	Na	Ne	I	He	UHe	% P
0	3	0.639 ± 0.109	1.167 ± 0.033	0.160 ± 0.029	0.105 ± 0.019	0.126 ± 0.023	30.56
10	3	0.833 ± 0.117	1.259 ± 0.041	0.230 ± 0.033	0.154 ± 0.023	0.184 ± 0.027	41.67
20	3	0.736 ± 0.114	1.198 ± 0.035	0.189 ± 0.030	0.124 ± 0.020	0.149 ± 0.024	36.11
30	3	0.944 ± 0.118	1.268 ± 0.039	0.251 ± 0.032	0.165 ± 0.022	0.189 ± 0.026	47.22
40	3	0.681 ± 0.110	1.188 ± 0.036	0.172 ± 0.030	0.144 ± 0.021	0.114 ± 0.021	31.94

He: Expected heterozygous allele; uHe: Unbiased Expected Heterozygosity; Na: Actual allele count; Ne: Effective allele count; and P: Polymorphic; I: Shannon's Information Index

Table 4 reveals that the population's allele frequency for all gamma-ray irradiation dose treatments (0, 10, 20, 30, and 40 Gy) is Na, with Na larger than Ne. This indicates that all populations at any irradiation dose have low diversity. The ratio of homozygous to heterozygous alleles demonstrates this limited diversity. The calculated heterozygous allele values for each gamma-ray irradiation dosage have lower He values than UHe. This suggests heterozygous alleles with low diversity. The percentage of polymorphism values in all gamma-ray irradiation dose groups ranges from 30 to 48%, demonstrating that gamma-ray exposure produces more homozygous alleles, both recessive and dominant, than heterozygous alleles. This suggests that gamma-ray dosages can cause genetic variety with a genetic distance related to a recombination frequency of 30–48%. Futhermore, a recombination frequency of 30-48% implies a difference of 30-48 cM (*centimorgans*) in genetic distance between the locus amongs individuls M1V1. However, this can also occur when primers recognize high annealing sites due to their structure, increasing the likelihood of detecting DNA polymorphisms among individuals [46].

Populations receiving a 30 Gy dosage produce the greatest Shannon's Information Index values. This suggests that populations with the highest diversity levels are obtained from populations given a dose of 30 Gy. Thus, the gamma-ray irradiation technique on *Solomon* teak plantlets can generate adequate diversity ranging from 30-48%. Additional, individuals treated with 30 Gy have the greatest amounts of Na (0.944 ± 0.118) and He (0.165 ± 0.022), while the lowest values are 0.639 ± 0.109 and 0.105 ± 0.019, respectively. It is suspected that gamma-rays have been able to increase the number of alleles and heterozygosity of each about 50% and 50%, respectively, is indicating low diversity.

3.5 Acclimatization of putative-mutant Solomon teak seedlings

Shoots regenerated from callus exhibited significant elongation by 35 weeks. These shoots were subsequently excised from the callus and acclimatized in a substrate composed of sand, compost, charcoal, and rice husk in a 1:1:2 (v/v/v) ratio to develop into seedlings. Plantlets derived from M1V1 callus regeneration

displayed somaclonal variation at the individual level. Ten teak seedlings are produced from dosages of 0 Gy, 10 Gy, 20 Gy, and 40 Gy by callus regeneration and acclimatization (Figure 5).

Putative-mutant seedlings obtained using irradiation techniques are still limited, thus they must be maintained and multiplied via tissue culture or shoot cutting techniques. These teak seedlings are kept and planted in the field to provide genetic variation for *Solomon* teak that can be utilized to assemble specific *Solomon* teak features. The root system of putative-mutant teak seedlings exposed to 20 Gy of gamma radiation is more developed than the root system of untreated teak seedlings. Teak seedlings grown without irradiation (*control*) grow faster than mutant teak seedlings grown at a dose of 20 Gy. Teak seedlings irradiated at doses of 20 Gy and 40 Gy are greener than non-irradiated seedlings and seedlings irradiated with a dose of 10 Gy, and they have two branches, showing the possibility for accelerating teak plant propagation. Mutagenesis (*physical and chemical*) induces low amounts of chlorophyll in *Delphinium malabaricum* mutants [47].

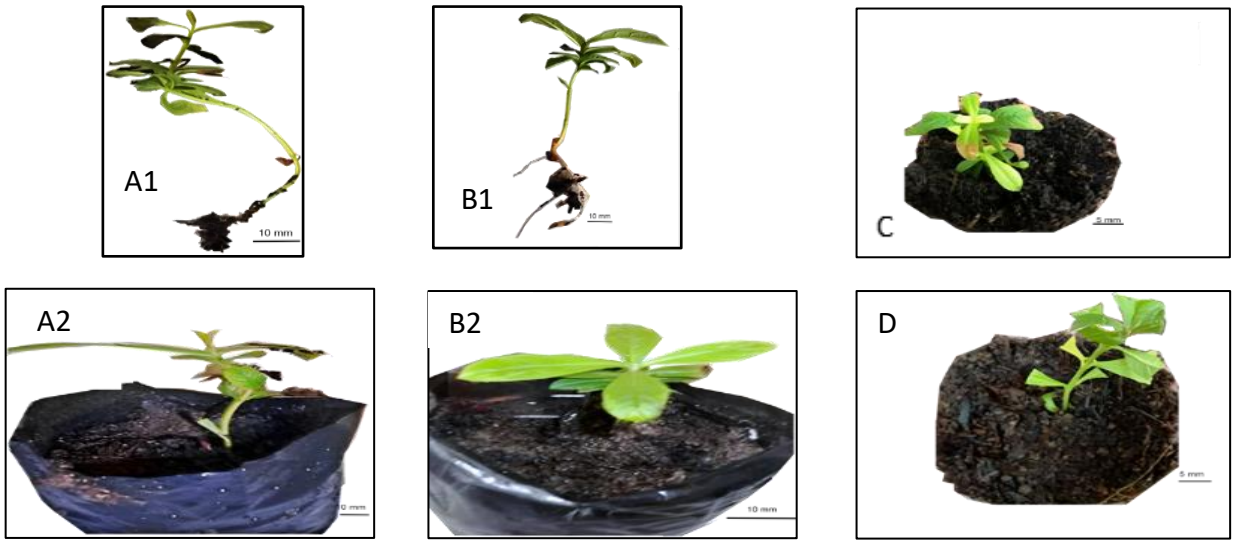

Figure 5. Performance of M1V1 seedlings exposed with gamma-rays three months after acclimation. Root system of non-irradiated teak seedlings (control) with scale 1: 10 (A1, A2), root system and performance of teak seedlings with 20 Gy irradiation dose with scale 1:10 (B1, B2), performance of teak seedlings with 10 Gy irradiation dosage with scale 1:5 (C), performance of teak seedlings with 40 Gy irradiation dose with scala 1:5 mm (D).

4.Conclusion

Gamma irradiation induces morphogenetic responses that may be utilized to determine effective and efficient dosage levels. Treatments with 10–40 Gy on MS medium supplemented with 0.1 ppm kinetin result in callus formation within two weeks, followed by the development of greenish-yellow embryogenic callus in subsequent weeks. Genetic analysis reveals that individuals across all treatments (0–40 Gy) exhibit a greater number of observed alleles than effective alleles (Na > Ne), and lower expected heterozygosity compared to the frequency of recessive homozygotes (He < UHe), indicating low genetic diversity. Notably, treatment with 30 Gy produces the highest values of Na (0.944 ± 0.118) and He (0.165 ± 0.022), suggesting that gamma irradiation can enhance allele number and heterozygosity by approximately 50%. Morphological alterations, including leaf withering and senescence, are more pronounced at higher doses (30–40 Gy). Although putative-mutant seedlings generated through irradiation remain limited in number, they can be preserved and propagated through tissue culture or shoot cutting techniques.

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