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GAMMA-IRRADIATION RESPONSE OF CASSAVA (VAR. UBI KUNING)

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ABSTRACT

Cassava (Manihot esculenta) is a key staple crop in tropical and subtropical regions, renowned for its ability to thrive in poor soils and withstand drought conditions. In Malaysia, cassava cultivation faces challenges such as low genetic diversity, susceptibility to pests and diseases, and limited yield improvement due to reliance on traditional breeding methods. Despite its economic importance, traditional breeding methods in cassava are challenged by complex polyploid genome and predominantly vegetative propagation. This research aimed to evaluate the acute radiosensitivity of cassava var. Ubi Kuning to gamma irradiation as a preliminary step toward enhancing genetic variability for mutation breeding programs. In this study, evaluation of the acute radiosensitivity of cassava cuttings to gamma irradiation, using doses of 0 (control), 5, 10, 15, 20, 25, 30, 40, 50, and 60Gy was conducted. The cuttings were irradiated with a gamma irradiator (Biobeam GM8000) using Caesium-137 as the source, and subsequently planted in polybags in a glasshouse. The survival data indicated an LD_{50} of approximately 33Gy whilst plant height data showed a GR_{50} of approximately 28Gy. The results show that as the gamma radiation dose increases, both data of the survival and growth of cassava decrease, demonstrating the plant's sensitivity to gamma irradiation. The findings suggest that doses around 28-33 Gy induced significant genetic variability while maintaining sufficient viability for mutation breeding programs. This study emphasizes the importance of optimizing irradiation doses as a foundation for developing improved cassava cultivars through mutation breeding.

Keywords: Acute gamma irradiation; mutation breeding; radiosensitivity test; cassava

ABSTRAK

Ubi kayu (Manihot esculenta) adalah tanaman makanan ruji utama di kawasan tropika dan subtropika, terkenal dengan kemampuannya untuk tumbuh di tanah yang kurang subur dan tahan kepada keadaan kemarau. Di Malaysia, penanaman ubi kayu menghadapi cabaran seperti kepelbagaian genetik yang rendah, kerentanan terhadap perosak dan penyakit, serta peningkatan hasil yang terhad akibat kebergantungan kepada kaedah pembiakbakaan tradisional. Di sebalik kepentingan ekonominya, pembaikbakaan ubi kayu adalah sukar kerana genom poliploid yang kompleks dan hanya dipropagasi secara vegetatif. Penyelidikan ini bertujuan untuk menilai radiosensitiviti akut keratan ubi kayu var. Ubi Kuning terhadap penyinaran gama sebagai langkah awal ke arah meningkatkan kepelbagaian genetik untuk *program pembiakbakaan mutasi. Dalam kajian ini, respon terhadap penyinaran gama menggunakan dos 0 (kawalan), 5, 10, 15, 20, 25, 30, 40, 50, dan 60Gy ke atas keratan ubi kayu dijalankan. Keratan ubi kayu disinarkan dengan sel gama (Biobeam GM8000) yang menggunakan Caesium-137 sebagai punca sinaran, dan seterusnya ditanam dalam polibeg di rumah kaca. Data kelangsungan hidup menunjukkan LD_{50} sekitar 33Gy manakala data ketinggian menunjukkan GR_{50} sekitar 28Gy. Keputusan menunjukkan bahawa apabila dos sinaran gama meningkat, kedua-dua data kelangsungan hidup dan pertumbuhan ubi kayu menurun, yang menunjukkan sensitiviti tanaman ini terhadap sinaran gama. Hasil dari eksperimen ini mencadangkan bahawa dos sekitar 28-33Gy adalah dos yang optima untuk digunakan bagi pembaikbakaan mutasi ubi kayu. Kajian ini menekankan kepentingan mengoptimumkan dos penyinaran sebagai asas untuk membangunkan kultivar ubi kayu yang lebih baik melalui pembiakan mutasi.*

Keywords: Penyinaran gama akut, pembaikbakaan mutasi, ujian radiosensitiviti, ubi kayu

INTRODUCTION

Cassava (*Manihot esculenta*) is a vital food crop, providing a primary source of calories for over 800 million people worldwide, particularly in sub-Saharan Africa, Latin America, and Southeast Asia (FAO, 2013). In Malaysia, cassava plays a significant role in both the agricultural and industrial sectors. Traditionally grown as a staple food crop, cassava has increasingly gained importance as a raw material for various industries, including starch production, animal feed, and bioethanol. The crop's ability to thrive in diverse agro-ecological conditions, including marginal soils and drought-prone areas, makes it a valuable resource for Malaysian farmers. Its adaptability to diverse agro-ecological conditions and resilience to abiotic stresses such as drought and poor soils make it a critical crop for food security and economic development in these regions (Nassar & Ortiz, 2010).

Cassava *var.* Ubi Kuning, or yellow cassava, is an economically significant crop in Malaysia, particularly in rural and agricultural communities. Known for its vibrant yellow flesh, Ubi Kuning is a source of income through its diverse applications in food processing and industrial uses. In Malaysia, this variety of cassava is valued for its nutritional richness and versatility, contributing to both food security and economic stability in regions where it is cultivated (Loh *et al.*, 2014). However, cassava production faces numerous challenges, including susceptibility to pests and diseases, and relatively low genetic diversity, which limits its potential for improvement through traditional breeding methods (Ceballos *et al.*, 2010). Recent efforts have focused on improving cassava varieties through breeding programs aimed at enhancing yield, disease resistance, and starch content, thus ensuring the crop's sustainability and economic viability in the face of growing industrial demand and changing climatic conditions (Malik *et al.*, 2020).

Conventional plant breeding, which relies on the natural genetic variation within a species, has been fundamental in improving crop varieties over the past century. However, the inherent genetic limitations and long breeding cycles of conventional methods often hinder the rapid development of new cultivars, especially in crops like cassava with long growth periods and limited genetic variability (Nassar & Ortiz, 2010). Mutation breeding, a technique that involves the induction of genetic mutations to create novel genetic variability, has emerged as a great tool for crop improvement (Ahloowalia *et al.*, 2004). This approach has been used since the early 20th century, with the first classic and successful mutation-induced crop variety being developed in the 1920s (Foster, 2013). Through the application of physical mutagens such as gamma rays and X-rays, or chemical mutagens like ethyl methanesulfonate (EMS) and sodium azide, plant breeders can induce mutations in seeds or other plant tissues to generate a wide range of genetic diversity (Shu *et al.*, 2012). In cassava, mutation breeding holds significant promise for overcoming its genetic bottlenecks and accelerating the development of superior cultivars (Nkere *et al.*, 2019).

The process typically involves exposing seeds or plant tissues to a mutagen, followed by the growth and screening of mutated populations for desirable traits. This method has led to the development of numerous improved crop varieties with enhanced traits such as disease resistance, improved nutritional content, and increased yield (Oladosu *et al.*, 2016). Notable examples include high-yielding wheat and rice varieties, disease-resistant barley, and improved oil composition in soybean (Ahloowalia *et al.*, 2004).

However, to start a mutation breeding program, radiosensitivity test which is crucial in mutation breeding need to be done as it helps to determine the optimal dose of radiation required for inducing mutations without causing excessive damage to the plant tissues. These tests involve exposing plant materials, such as seeds, cuttings, or tissue cultures, to a series of doses of irradiation and assessing their responses in terms of survival rate, growth inhibition, and frequency of mutations (Roychowdhury & Tah, 2011). The results of radiosensitivity tests guide breeders in selecting the appropriate irradiation dose that maximizes genetic variability while minimizing lethality and detrimental effects. For instance, the lethal dose (LD_{50}), which is the irradiation dose at which 50% of the treated population is expected to die, is a critical parameter often determined during the test (Mustapha *et al.*, 2019). Additionally, sublethal doses that induce a significant mutation rate without severely affecting plant viability are identified to optimize mutation breeding efforts.

This paper aims to study the response of cassava *var.* Ubi Kuning against acute gamma irradiation to determine its optimal dose for subsequent mutation induction work in the future.

PROCEDURE

Plant material

Cassava *var.* Ubi Kuning cuttings were sourced from Taman Kekal Pengeluaran Makanan (TKPM), Ulu Chuchoh, Sepang Selangor. For consistency and reliability in the study, only cuttings with a diameter of 5cm and a length of 15cm were selected. These cuttings were used to ensure uniformity in the experiment and to facilitate accurate comparisons across different irradiation treatments.

Experimental design and irradiation of cuttings

The experiment was conducted using a Completely Randomized Design (CRD) to evaluate the effects of gamma irradiation on cassava cuttings. The cuttings were subjected to gamma irradiation using a gamma irradiator (BioBeam GM 8000, Germany) at Nuklear Malaysia, with Caesium-137 as the radiation source. A range of gamma irradiation doses was applied to the cuttings, specifically 5, 10, 15, 20, 25, 30, 40, 50, and 60 Gy. A control group was included, which consisted of cuttings that were not exposed to gamma irradiation. Each dose and the control have three (3) biological replicates and ten (10) technical replicates to ensure the reliability and reproducibility of the results.

Soil preparation and cuttings planting

Mix organic soil containing topsoils, coconut and rice husk, compost and microbial fertilizer was filled in 12x15 inches polybags and prepared with the same total number of irradiated and control cuttings. Each irradiated and control cuttings was planted in the polybags at the glasshouse in slanted mode and watered every two days.

Data collection and analysis

Data collection were carried out at eight weeks after planting. Key parameters measured included survival rate, plant height, number of leaves, and number of buds. Additionally, leaf morphology was documented to assess the effects of gamma irradiation on leaf structure. Statistical analysis was performed using Analysis of Variance (ANOVA) with Statistical Analysis System (SAS) version 9.2 to determine significant differences ($p \leq 0.001$) among the various treatment groups. The Least Significant Difference (LSD) test was employed to assess mean differences between irradiation doses. For radiosensitivity analysis, the best-fit model from Curve Expert 1.3 was used to estimate the LD_{50} (lethal dose for 50% of the population) and GR_{50} (growth reduction dose for 50% of the population).

RESULTS AND DISCUSSION

Lethal Dose 50

The percentage of survived plants (survival rate) of cassava under different gamma irradiation treatments was investigated. The number of survived plants was scored after 8 weeks of planting in order to determine the Lethal Dose 50 (LD_{50}). The treatments ranged from 0Gy to 60Gy, with significant differences observed among the groups as illustrated in Table 1. Specifically, treatments sharing the same letter (e.g., a) in the t Grouping are not significantly different from each other, while treatments with different letters represent significantly different survival rates. From the results obtained, treatments with 10Gy and 20Gy showed similar survival rates with control which is 0.9 or 90% and were not significantly different from each other. The highest survival rate was observed in the 15Gy treatment, which had a mean survival rate of 1.0 which is 100% of survival. This suggests that these doses are within a tolerable range for cassava, allowing high survival rates. Similar findings have been reported in previous studies where moderate doses of gamma irradiation have been shown to not causing significant harm to the population (Mba *et al.*, 2010).

Table 1. Cassava *var.* Ubi Kuning survival rate at 8th week after planting

Dose (Gy)	Survival rate (%) (Means)	t Grouping		
0 (Control)	90	b	a	
5	80	b	a	c
10	90	b	a	
15	100		a	
20	90	b	a	
25	70	b		c
30	60			c
40	10		d	
50	0		d	
60	0		d	

However, a notable drop in survival rate was observed in the 5Gy treatment group with 80% of survival rate. Although this dose is lower than the aforementioned groups, it resulted in a statistically significant decrease in survival rate, indicating a possible threshold for beneficial effects of low-dose gamma irradiation. Lower survival rates at this dose may be due to the activation of stress responses in the plants. The survival rates continued to decrease with increasing doses of irradiation, with the 25Gy and 30Gy treatments having mean survival rates of 0.7 or 70% and 0.6 or 60%, respectively. These doses appear to be approaching the upper limit of cassava's tolerance to gamma irradiation. This trend aligns with other studies where higher doses of gamma irradiation have been shown to cause detrimental effects on plant health, such as reduced growth and increased mortality (Kodym & Afza, 2003).

The most severe reductions in survival rates were observed at 40Gy with only 10% of survival rate whilst 50Gy and 60Gy showed no survival. These results demonstrate that high doses of gamma irradiation are lethal to cassava, causing significant damage that the plants cannot recover from. This finding is consistent with established study on the effects of high-dose radiation on plants, which can lead to cellular damage and death (Kim *et al.*, 2019).

The fitted curve extracted from Curve Expert 1.3 for survival rate against dose as shown in Fig. 1, with a high correlation coefficient (r = 0.977), indicates a strong fit to the observed data, suggesting the model accurately represents the relationship between gamma irradiation doses and cassava survival rate. The LD_{50} which is the dose at which 50% of the cassava plants survive in comparison with control population, was estimated to be around 32Gy. This value indicates the dose at which half of the cassava plants do not survive, providing a benchmark for safe and effective doses in mutation breeding programs for cassava *var*. Ubi Kuning. The information obtained from this result can guide the application of gamma irradiation in cassava breeding, ensuring the selection of doses that maximize mutation induction while minimizing plant mortality.

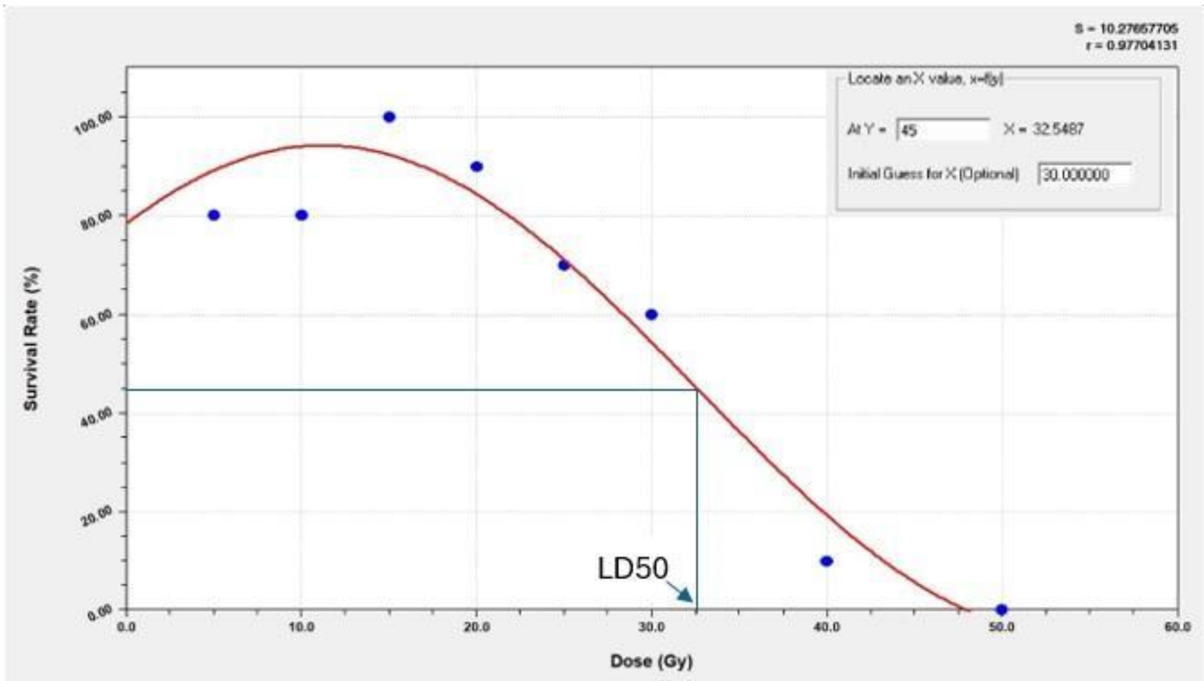

Figure 1. Survival rate (%) of irradiated cassava and control against dose (Gy) at 8th week after planting

Growth Reduction Dose 50

Growth reduction dose 50 (GR_{50}) can be calculated from the plant height data. The statistical analysis revealed significant differences in plant height among the various treatment groups as shown in Table 2. Treatments assigned the same letter (e.g., a) in t Grouping indicate no significant difference in plant height, whereas treatments assigned different letters represent statistically significant differences in plant height. The highest mean plant height was observed in the 15Gy treatment group which is 86.1cm, followed closely by the 10Gy treatment group (82.4cm), with both groups showing no significant difference from each other but has significant different in comparison with control. Even though treatment at 5Gy and 20Gy has no significant different compared to control, but the means of plant height treated with both doses is a bit high in comparison with control. This is consistent with findings in other mutation breeding studies where low to moderate doses of gamma radiation have been reported to enhance growth traits in various crops such as reported by Jaipo *et al.* (2019). A classic study by Pitirmovae (1979) mentioned that stimulation of cell division can affect the synthesis of nucleic acid at low dose of gamma irradiation that might rouse the growth of plant.

Table 2: Cassava *var.* Ubi Kuning plant height (cm) at 8th week after planting

Dose (Gy)	Plant height (cm) (Means)	t Grouping	
0 (Control)	54.7	c	
5	57.6	c	
10	82.4	a	b
15	86.1	a	
20	68.4	c	b
25	22.6	d	
30	18.9	d	
40	1.0		e
50	0		e
60	0		e

Furthermore, as irradiation dose increased from 25Gy to 60Gy, plant height drastically decreased, with the 40Gy resulting in mean plant heights of 1.0cm only whilst both 50Gy and 60Gy treatments killed the population. These high-dose groups demonstrated low plant height, aligning with previous research that highlights the detrimental effects of high gamma radiation doses on plant development as mentioned by Omar *et al* (2008) where DNA damage and inability to repair them is the reason of the severe growth inhibition or complete lethality.

Similarly with LD_{50}, growth reduction dose (GR_{50}) refers to the growth reduction by 50%, which is a measure used to determine the dose of a mutagen that reduces the growth of a plant growth e.g. plant height, number of leaves, number of buds etc by 50%. As illustrated in Fig. 2, the GR_{50} for cassava based on plant height data was determined to be approximately 28Gy, providing a critical reference point for optimizing gamma irradiation doses in cassava *var.* Ubi Kuning. As mentioned above in the LD_{50} discussion, this value underscores the plant's relative tolerance to radiation and highlights the importance of carefully selecting irradiation doses to balance mutagenic effectiveness with plant viability.

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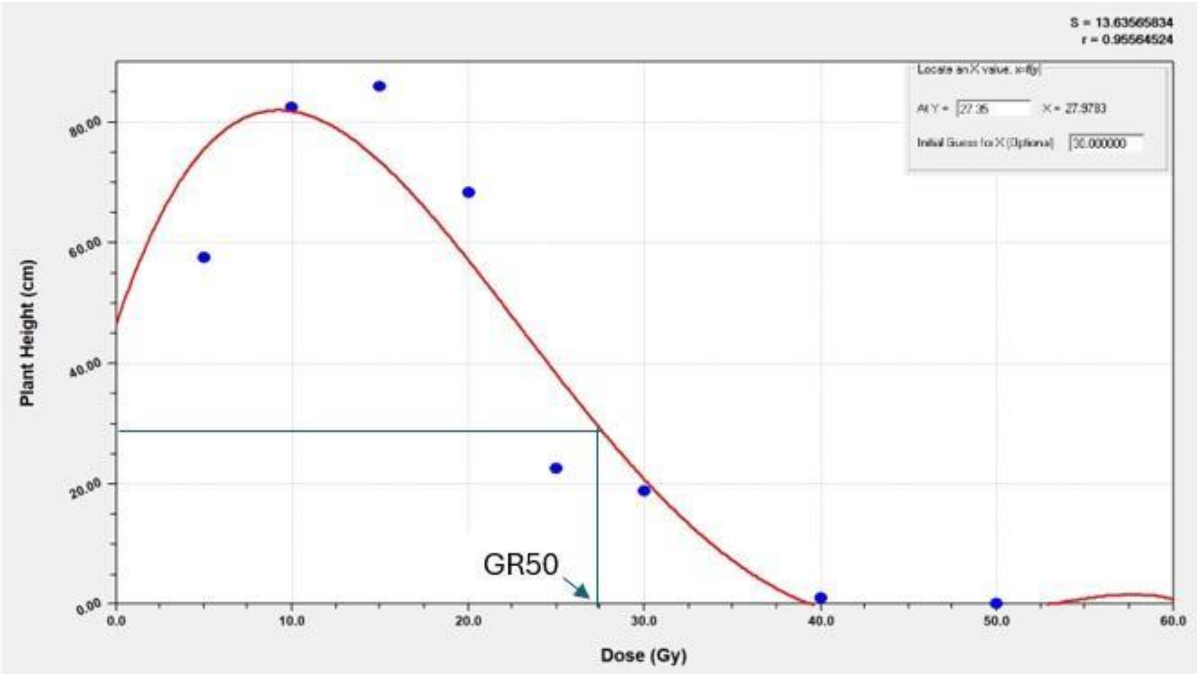

Figure 2. Plant height (cm) of irradiated cassava and control against dose (Gy) at 8th week after planting

Effect of acute gamma irradiation on number of shoots and number of buds of cassava *var.* Ubi Kuning

The impact of gamma radiation on cassava plants demonstrates significant effects on both the number of leaves and number of buds. Treatments labeled with the same letter (e.g., a) in t Grouping denote no statistically significant differences in the number of leaves, while those labeled with different letters indicate significant variations in leaf count. For number of leaves as shown in Table 3, the control group (0Gy) and lower dose groups i.e. 5Gy, 10Gy, and 15Gy displayed comparable leaf numbers, with the population irradiated with 15Gy exhibiting the highest mean which is 7.8. This suggests that low doses of gamma radiation might not adversely affect, and could even slightly enhance leaf development. However, as the radiation dose increased beyond 20Gy, there was a notable decline in leaf numbers. At 40Gy the mean leaf numbers dropped drastically to 0.4, whilst doses above 40Gy showed no development of leaves, indicating severe damage to the plant's ability to produce leaves due to high levels of irradiation (Kovalchuk *et al.*, 2003; Kim *et al.*, 2010).

Table 3. Number of leaves of cassava *var.* Ubi Kuning at 8th week after planting

Dose (Gy)	Number of leaves (Means)	t Grouping		
0 (Control)	7.3	b	a	
5	7.2	b	a	
10	7.1	b	a	
15	7.8		a	
20	6.4	b	a	c
25	4.6	b		c
30	3.7			c
40	0.4		d	
50	0		d	
60	0		d	

Similarly, the data on the number of buds in cassava *var.* Ubi Kuning also reveals a pronounced effect of acute gamma irradiation as illustrated in Table 4. Treatments sharing the same letter (e.g., a) in t Grouping indicate no statistically significant differences in buds count, whereas treatments assigned different letters represent significant differences in the number of buds. The control group (0Gy) had a mean bud number of 1.9. Low irradiation doses up to 20Gy did not significantly reduce the bud number, with the 15Gy treatment showing the highest mean of bud number which is 3.0. This indicates a potential stimulatory effect of low-dose radiation on bud development (Wang *et al.*, 2007). However, higher doses of irradiation which were 25Gy and above, led to a significant reduction in bud numbers. At 40Gy, the mean of number of buds decreased drastically to 0.1 and similarly with number of leaves, no formation of buds was observed at 50Gy and 60Gy due to the higher irradiation dose that can affect the cell growth.

Table 4. Number of buds of cassava *var.* Ubi Kuning at 8th week after planting

Dose (Gy)	Number of buds (Means)	t Grouping		
0 (Control)	1.9	b	d	c
5	1.9	b	d	c
10	2.6	b	a	
15	3.0		a	
20	2.2	b	a	c
25	1.5		d	c
30	1.3		d	
40	0.1		e	
50	0		e	
60	0		e	

These observations highlight the dual effects of gamma radiation on cassava agronomic characteristics. Low doses may promote certain growth parameters, such as leaf and bud development, while higher doses generally inhibit growth due to cellular damage. This information is crucial for optimizing gamma irradiation doses in mutation breeding programs aimed at enhancing cassava traits.

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Leaves morphology

The impact of gamma irradiation on leaf morphology varies across different plant species and doses applied. Leaves play a crucial role in the life of a plant, serving as the primary sites for photosynthesis, the process by which plants convert light energy into chemical energy stored in glucose. This function is essential for the growth and energy needs of the plant. Leaves are also vital for gas exchange, containing stomata that open and close to regulate the intake of carbon dioxide and the release of oxygen and water vapor. Changes to leaf morphology can have significant implications for a plant's overall health and functionality. Generally, gamma irradiation can cause a range of morphological changes in leaves, including alterations in leaf size, shape, colour, and structure. In this study, various changes on the leaf's morphology of cassava *var*. Ubi Kuning were observed.

Control leaves 5Gy – Extra lobes of leaves 5Gy – Leaves abnormality

Figure 3. Effect of gamma irradiation on leaf morphology

As illustrated in Fig. 3, cassava plants subjected to a 5Gy gamma irradiation treatment exhibited a notable increase in the number of leaves compared to the control group, which had only five lobes. The irradiated plants not only had a higher leaf count but also demonstrated robust and healthy leaf development. This observation suggests a stimulatory effect of low-dose gamma irradiation on leaf production. The increased leaf number can be attributed to the activation of growth-promoting genes and hormonal changes induced by the irradiation. At lower doses, gamma irradiation has been known to enhance leaf area and improve photosynthetic activity, leading to overall better plant vigor and productivity (Kovács & Keresztes, 2002).

Despite these positive effects, it is important to note that some of the irradiated leaves exhibited irregular and distorted shapes as shown in Fig. 3 as well. This irregularity indicates that while low-dose gamma irradiation can enhance leaf production, it may also induce unintended morphological abnormalities. These abnormalities may arise due to the inherent randomness of mutation and the plant's repair mechanisms. The variability in response to irradiation underscores the complexity of mutation breeding, where beneficial effects are sometimes accompanied by adverse changes in plant morphology.

On the other hand, higher doses of gamma irradiation presented more pronounced detrimental effects on leaf morphology. As shown in Fig. 4, plants exposed to higher irradiation doses exhibited reduced leaf size, chlorophyll degradation, and necrosis (death of leaf tissue). These changes are indication of severe damage to cellular structures, including chloroplasts, which are essential for photosynthesis. Higher doses of gamma irradiation can inflict significant damage to DNA, proteins, and lipids, leading to cell death and tissue necrosis. The impairment

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of chloroplast function further contributes to decreased chlorophyll content, which ultimately hinders the plant's ability to produce energy and grow effectively (Wi *et al.*, 2007).

The observed effects underscore the dose-dependent nature of gamma irradiation. While low doses can positively influence plant growth and morphology by promoting leaf production, higher doses can have adverse impacts by disrupting critical physiological processes. The extent of these changes is influenced by the species' sensitivity to radiation and the specific dose administered. Understanding this dose-response relationship is crucial for optimizing gamma irradiation protocols in plant mutation breeding to balance beneficial outcomes with the potential risks of morphological and physiological damage.

Control leaves

10 Gy – Chlorophyll degradation

15 Gy – Crinckle leaves

20 Gy – Chlorophyll degradation

25 Gy – Leaf abnormality

Figure 4. Leaf mutation observed in irradiated cassava *var*. Ubi Kuning at different doses compare to the Control

CONCLUSION

In conclusion, this study demonstrates the acute radiosensitivity of cassava *var*. Ubi Kuning to gamma irradiation, with significant implications for mutation breeding program. The determined LD50 of approximately 33Gy and GR50 of approximately 28Gy provide critical benchmarks for the optimal irradiation doses that induce genetic variability while maintaining sufficient plant viability. Thus, doses between 28-33Gy can be used for mutation induction. The results indicate a clear dose-dependent response, where survival and growth rates decrease with increasing gamma radiation doses, highlighting the importance of carefully selecting appropriate doses to balance mutagenic effectiveness with plant health. These findings contribute to the broader understanding of radiation-induced mutagenesis in cassava, offering valuable insights for developing improved cultivars through mutation breeding.

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