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EFFECTS OF CHRONIC GAMMA IRRADIATION ON CASSAVA VAR. *UBI KUNING*

KESAN PENYINARAN GAMA KRONIK TERHADAP UBI KAYU VAR. *UBI KUNING*

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ABSTRACT

Cassava (Manihot esculenta Crantz) is one of the most essential carbohydrate crops worldwide, consumed by millions of populations in Africa, Asia, and Latin America (Parmar *et al.*., 2017). Cassava (Manihot esculenta Crantz) is one of the most essential carbohydrate crops worldwide, consumed by millions of populations in Africa, Asia, and Latin America (Parmar *et al.*., 2017). Cassava (Manihot esculenta Crantz) is one of the most essential carbohydrate crops worldwide, consumed by millions of populations in Africa, Asia, and Latin America (Parmar *et al.*., 2017). *Cassava, known scientifically as Manihot esculenta is one of the most essential carbohydrate crops worldwide, consumed by millions of populations in Africa, Asia, and Latin America. In Malaysia, Cassava produces various food items, including tapioca pearls, and cassava chips. Over the past two decades, advancements in mutation breeding techniques have significantly contributed to the genetic improvement of cassava, addressing challenges such as low yield, disease susceptibility, and poor nutritional content. Additionally, mutation breeding has facilitated the enhancement of root quality, including higher starch content and improved cooking properties. Cassava stem cuttings were exposed to various gamma irradiation dose rates (0.07 to 2.67 Gy/hour) over eight weeks. The results showed that survival rates remained high across most treatments, indicating cassava's resilience to radiation. However, plant height and bud formation were adversely affected by higher doses, with the highest cumulative dose (1709.49 Gy) resulting in the lowest plant height (30.4 cm) and reduced bud counts. Morphological changes, such as chlorophyll degradation and leaf abnormalities, were prevalent at higher doses. This suggests that while cassava can tolerate significant gamma irradiation, substantial doses impede growth and induce notable morphological mutations. Future research should focus on the growth of subsequent mutant generations to further understand the long-term impacts of chronic gamma irradiation on cassava.*

Keywords: Cassava var. Ubi Kuning, chronic gamma irradiation, mutation breeding, dose rate

ABSTRAK

Ubi kayu, yang dikenali secara saintifik sebagai Manihot esculenta, adalah salah satu tanaman karbohidrat yang paling penting di seluruh dunia, dimakan oleh berjuta-juta penduduk di Afrika, Asia, dan Amerika Latin. Di Malaysia, ubi kayu menghasilkan pelbagai produk makanan, termasuk bebola ubi kayu (tapioca pearls) dan kerepek ubi kayu. Sepanjang dua dekad yang lalu, kemajuan dalam teknik biakbaka mutasi telah menyumbang dengan ketara kepada peningkatan genetik ubi kayu, menangani cabaran seperti hasil yang rendah, kerentanan terhadap penyakit, dan kandungan nutrisi yang rendah. Selain itu, biakbaka mutasi juga telah membantu meningkatkan kualiti akar, termasuk kandungan kanji yang lebih tinggi dan sifat memasak yang lebih baik. Keratan batang ubi kayu telah didedahkan kepada pelbagai kadar dos sinaran gamma berbeza (0.07 hingga 2.67 Gy/jam) selama lapan minggu. Hasil kajian menunjukkan kadar kelangsungan hidup kekal tinggi dalam kebanyakan rawatan, menunjukkan ketahanan ubi kayu terhadap sinaran. Walau bagaimanapun, ketinggian tumbuhan dan pembentukan tunas terjejas oleh dos yang lebih tinggi, dengan dos kumulatif tertinggi (1709.49 Gy) menghasilkan ketinggian tumbuhan terendah (30.4 cm) dan bilangan tunas yang berkurang. Perubahan morfologi, seperti degradasi klorofil dan pembentukan daun yang tidak normah adalah lazim pada dos yang lebih tinggi. Ini menunjukkan bahawa walaupun ubi kayu dapat bertahan pada sinaran gamma yang tinggi, dos yang tinggi menghalang pertumbuhan dan menyebabkan mutasi morfologi yang ketara. Penyelidikan pada masa hadapan perlu fokus pada pertumbuhan generasi mutan seterusnya untuk memahami kesan jangka panjang sinaran gamma kronik pada ubi kayu.

Kata kunci: Ubi kayu var *Ubi Kuning*, Penyinaran gama kronik, Biakbaka mutasi, kadar dos

INTRODUCTION

Cassava (*Manihot esculanta*) also locally known as *ubi kayu* is a perennial woody shrub with edible roots, grown worldwide in tropical and subtropical climates (Amarullah *et al.* 2017; Oliveira and Miglioranza, 2014). Cassava is a vital local food source with a broad distribution and significant agroecological adaptability, making it the third most important tropical crop (Adiele *et al.* 2020; Neves *et al.* 2018; Hasibuan & Nazir, 2017). This versatile crop, a mesophyte, thrives in various environments, including fallow highlands, hill slopes, rice fields, and other underutilized highlands. This crop offers substantial development potential by providing essential calories and supporting food security for the growing population, particularly through local wisdom practices (Sulistiono *et al.* 2020; De Souza *et al.* 2017). Notably, cassava can grow in poor soils and still produce a reasonable yield, providing essential food security where other crops may fail (Siritunga *et al.* 2004). The ability to harvest cassava roots throughout the year ensures a reliable food supply for smallholder farmers and supplies raw materials for various processing industries (Rahman & Awerije, 2016).

According to the Food and Agriculture Organization of the United Nations (FAOSTAT, 2011), global cassava production exceeds 230 million metric tonnes annually. The leading producers include Nigeria, which yields approximately 37.5 million tonnes per year, Brazil with 24.5 million tonnes, and Thailand at 22.0 million tonnes. In Malaysia, the Department of Agriculture reported that 2023 around 2,815 hectares were dedicated to cassava cultivation, resulting in a production of about 46,156 metric tonnes (Department of Agriculture, 2023). Cassava has traditionally been used as a starch source in Malaysia (Tan & Khatijah, 2000). As understanding of the crop's potential expanded, the focus shifted to selecting cassava clones with lower cyanogen levels in the roots, making them suitable for direct consumption and food processing. Popular processed cassava foods include chips and crackers which are traditional snacks. Farmers currently cultivate two or three cassava varieties for chip production, including *Medan*, *Ubi Kuning*, and *Ubi Putih*.

Cultivation of cassava in Malaysia features a limited variety of both traditional and improved cultivars. While local traditional varieties have been cultivated for generations primarily for household consumption, they often face challenges such as susceptibility to pests, diseases, and generally low yields (Zainuddin *et al.* 2018). Improved varieties developed by the Malaysian Agricultural Research and Development Institute (MARDI), such as 'Sri Pontian' and 'Gajah', offer higher yields and better resistance to pests and diseases but are still limited in number compared to the extensive varietal diversity found in major cassava-growing regions like Africa and South America (Rahman *et al.* 2020). Despite these improvements, cassava farmers continue to struggle with common issues such as cassava mosaic disease (Patil and Fauquet, 2009), and root rot (Hohenfeld *et al.* 2022), which significantly impact production and profitability. Ongoing research and development efforts aim to enhance the existing cultivar pool and address these challenges to improve the overall productivity and sustainability of cassava cultivation in Malaysia.

Cassava varieties have a sterile genotype or produce highly heterozygous seeds with poor flowering which significantly hampers breeding efforts and genetic improvement of the crop. Sterile genotypes are unable to produce viable seeds, which restricts the generation of new genetic combinations through sexual reproduction. This leads to limited genetic diversity and reduces the potential for developing improved varieties with desirable traits such as increased yield, disease resistance, and environmental adaptability (Koundinya & Ajeesh 2023). Additionally, high heterozygosity and poor flowering complicate the breeding process, as it becomes challenging to select and stabilize traits over successive generations. The reliance on vegetative propagation in such cases, while effective for maintaining specific genotypes, limits the introduction of new genetic variations and slows down the overall breeding progress.

Mutation breeding has been a recognized method for creating new plant varieties with beneficial traits for a long time. Since the initial release of a mutant variety in the 1930s, the field has seen numerous notable achievements. A total of 3,308 new plant varieties have been developed globally through mutation breeding and have been registered with the International Atomic Energy Agency since 2019. Gamma irradiation has been widely used in plant breeding as a mutagenic agent to induce genetic diversity and improve traits like yield, disease resistance, and nutritional content (Ntsefong *et al.* 2023) However, the specific effects of chronic gamma irradiation on *Ubi Kuning*, particularly regarding its growth, development, and bud formation, are not well-documented. Previous research indicates that gamma irradiation can lead to cellular damage and morphological changes in plants (Wi *et al.* 2007), but the impacts on cassava specifically have not been extensively studied This lack of information limits the effective use of gamma irradiation for cassava improvement.

This study hypothesizes that chronic gamma irradiation will cause genetic mutations in *Ubi Kuning*, resulting in both positive and negative changes in its agronomic traits. It is expected that while certain doses of gamma irradiation may enhance traits such as disease resistance and nutritional content, they may also negatively impact growth parameters, including bud formation and shoot development. The procedure involved exposing cassava stem cutting to various dose rates of 0.66 Gy/hour (ring 2), 0.3 Gy/hour (Ring 3), 0.17 Gy/hour (Ring 4), 0.11 Gy/hour (Ring 5), 0.07 Gy/hour (Ring 6), and 0 Gy/hour (control) and observations were made on plant survival and morphological characteristics such as plant height, number of buds, and phenotypic/morphological variation. Therefore, this study was conducted to investigate the effects of chronic gamma irradiation on the growth and morphological changes in *Ubi Kuning*.

PROCEDURE

Plant Materials and preparation

Ubi Kuning stem cutting was obtained from Taman Kekal Pengeluaran Makanan (TKPM) Ulu Chuchoh, Sepang, Selangor. A total of 70 stem cuttings were used in this study. The stem cutting was chosen based on their diameter approximately five cm in diameter and was cut into 15 cm lengths each. The experiment was conducted using a completely randomized design (CRD) with seven treatments which are control, isodose ring 1,2,3,4,5, and 6 with 10 replicates for each treatment.

Chronic gamma irradiation exposure

A total of 60 *Ubi Kuning* stem cuttings were exposed to chronic gamma irradiation in the Gamma Greenhouse (GGH) facility in the Malaysian Nuclear Agency. The stem cuttings were placed in pots at 6 different dose rates ranging from 0.07 Gy/hour, 0.11 Gy/hour, 0.17 Gy/hour, 0.3 Gy/hour, 0.66 Gy/hour, and 2.67 Gy/hour. As for control treatment, a total of 10 stem cuttings were grown outside the gamma house under conditions very similar to those irradiated plants.

Data collection

Data on Plant Survival, plant height, number of buds, and morphological changes of the plants were observed during the experiment. Plants were taken out from GGH at Week 8 after planting and an accumulated dose of gamma irradiation for each treatment was collected at the end of the experiment.

Statistical Analysis

Analysis of variance was performed using Statistical Analysis System (SAS) version 9.2 to measure significant differences ($p \leq 0.05$) among characteristics data. The mean difference between doses was further tested using the least significant difference (LSD) method at 5%.

RESULTS AND DISCUSSION

Stem cuttings of *Ubi Kuning* were subjected to chronic gamma irradiation for 8 weeks (32 days) to study the plant's response to varying doses of gamma rays. Gamma rays, known for their high penetration ionizing radiation, are utilized as mutagens in plant mutation breeding. Chronic induced mutation from GGH can be used to irradiate different plant materials, including seedlings in pots, cuttings, calluses, somatic embryos, and suspension cell cultures (Nur Aziliana *et al.* 2015; Azhar, 2009). A previous study has reported that mutagenic treatment significantly affects plant survival rates and morphological traits (Harding and Mohamad, 2009; Mohamad *et al.* 2002). The data on cumulative dose is presented in Table 1.

Table 1: Cumulative dose

Isodose Ring	Dose Rate (Gy/hr)	Accumulative dose (Gy)
Control	0	0
1	2.67	1709.49
2	0.66	450.58
3	0.30	192.8
4	0.17	109.25
5	0.11	70.69
6	0.07	44.99

The survival rates of cassava under various treatment conditions are shown in Figure 1. The accumulated doses range from 0 to 1709.49 Gy, while the survival rate of *Ubi Kuning* at week 8 predominantly remains at 100%, except for a slight dip to 90% at an accumulated dose of 44.99 Gy. This suggests that the accumulated dose does not significantly impact the survival rate at week 8, maintaining a consistent level of 100% regardless of the dose, except for the minor deviation at the 44.99 Gy dose. This consistency indicates a potential threshold effect or resistance to change in response to increasing doses. Chronic irradiation differs significantly from acute gamma irradiation. Acute irradiation involves exposing samples to a high dose of radiation over a short period, whereas chronic gamma irradiation continuously exposes samples to low doses of radiation over an extended period. Theoretically, during chronic exposure, plant DNA undergoes continuous breakdown and repair, leading to mutations that help the plant survive (Mohd Zulmadi et al). This ongoing adaptation process might explain why the survival rate is not significantly affected by varying doses of gamma radiation.

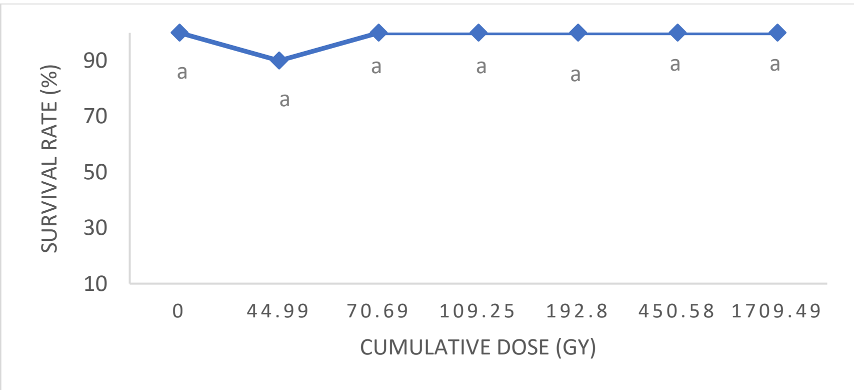

Figure 1: Effect of chronic gamma irradiation on the survival rate of *Ubi Kuning* at 8 weeks after planting

*Means with different letters between treatments indicate statistically significant differences at P≤0.05

Plant size and plant height have been reported to be affected by the dosage of mutagen received (Baadu *et al.* 2023). Figure 2 illustrates the impact of cumulative dose (Gy) on the height of *Ubi Kuning* plants measured at week 2 and week 8. Initially, at week 2, plant height increases with cumulative doses of 44.99 Gy, 70.69 Gy, and 109.25 Gy peaking at 12.25 cm. However, higher doses such as 192.8 Gy and 450.58 Gy result in significantly reduced heights of 8.9 cm and 6.1 cm, respectively compared to lower cumulated doses. At the extreme dose of 1709.49 Gy, the height further drops to 5.67 cm. Similarly, at week 8, the highest cumulative dose of 1709.49 resulted in the lowest plant height of 30.4 cm which is significantly different from other doses. The data suggest that very high doses of radiation are ultimately detrimental to the plant's height. Similarly, a significant reduction in plant height is also reported with higher doses of radiation in the *Chrysanthemum morifolium* variety 'otome pink' (Kumari *et al.* 2013). This radiation injury could be due to the inhibition of DNA synthesis or other physiological damage that not just appeared in plant height but could also be manifested in the form of plant survival and the number of plant organs (Nwachukwu, *et al.* 2009). Besides, Tiwari and Kumar (2011) also reported that many mutations can be lethal caused by the inhibition of cell division and induction of cell death. In some crops, lower plant height can be useful as it can withstand strong wind. Therefore, it does not mean that lower plant height cannot be selected for further screening.

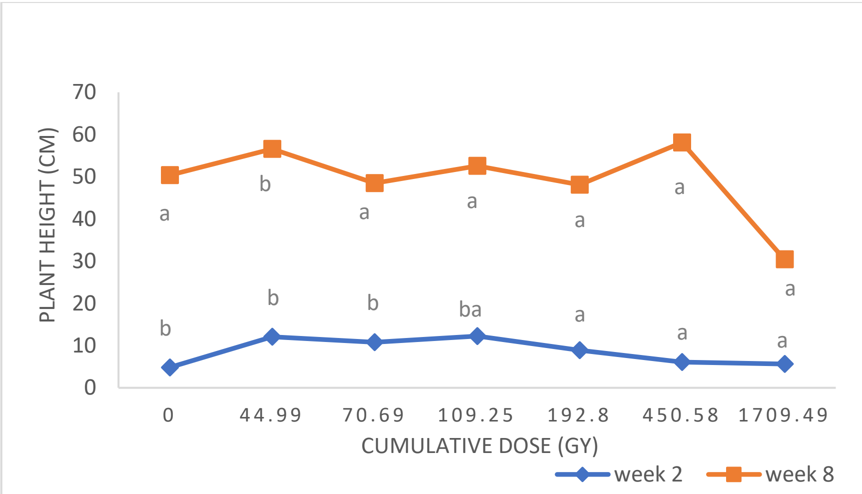

Figure 2: Effect of chronic gamma irradiation on plant height of *Ubi Kuning* at week 2 and week 8 after planting

*Means with different letters between treatments within the same week indicate statistically significant differences at P≤0.05

Figure 3 reveals the effect of cumulative doses (Gy) on the number of buds in *Ubi Kuning* plants over two time periods: week 2 and week 8. Initially, at week 2, the number of buds shows slight variations with cumulative doses, ranging from 3.0 to 3.6 buds. The control group (0 Gy) and the highest dose (1709.49 Gy) both exhibit 3.4 buds, indicating no immediate impact from extreme radiation levels. The highest bud count at week 2 is observed at 70.69 Gy with 3.6 buds, while the lowest is at 44.99 Gy with 3 buds with no significant difference between the dose rates. However, by week 8, there is a noticeable decline in the number of buds across all doses. The control group maintains the highest number of buds at 3.4, while all other groups show reduced bud counts, ranging from 2.4 to 2.7 buds. The highest dose of 1709.49 Gy results in a bud count of 2.5, similar to the mid-range doses of 192.8 Gy and 450.58 Gy. This data suggests that while radiation exposure may not drastically affect bud count in the short term, it leads to a consistent reduction in bud formation over an extended period. The number of buds can affect the number of leaves. Leaves are plant organs that perform photosynthesis, which produces carbohydrates for plants (Gardner *et al.* 2017). A previous study conducted by Hartati *et al.* 2021 showed that the low number of buds has a significant to very significant effect on plant height, stem diameter, and number of leaves. This is possible because as the number of buds increases, the nutrients produced from photosynthesis will be divided and distributed into many buds, resulting in decreased plant growth and yield.

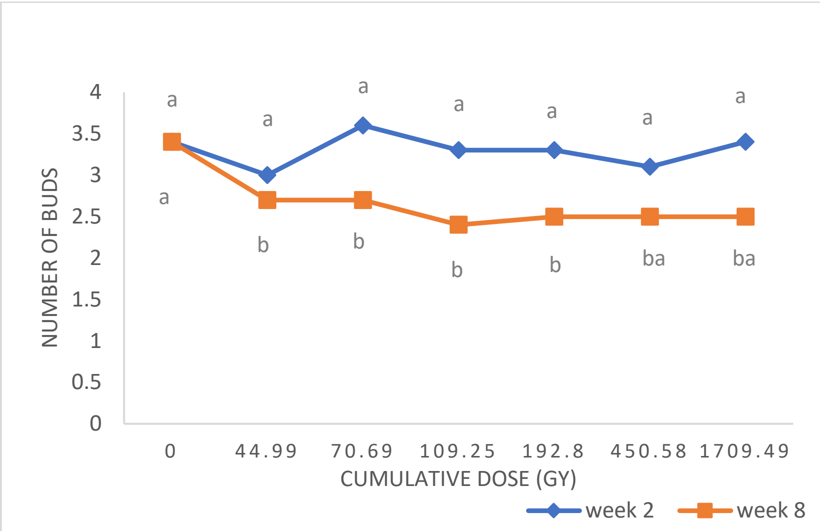

Figure 3: Effect of chronic gamma irradiation on the number of buds of *Ubi Kuning* at week 2 and week 8 after planting

*Means with different letters between treatments within the same week indicate statistically significant differences at P≤0.05

The study observed significant morphological changes in cassava plants exposed to chronic gamma radiation, with variations dependent on the dose level. Table 2 and Fig. 4a, 4b, and 4c show the morphological changes that can be observed at week 8 after planting for each isodose ring. At eight weeks after planting, control plants displayed normal coloration and typical leaf morphology with five lobes. In contrast, plants in all isodose rings exhibited chlorophyll degradation and various leaf abnormalities, including altered leaf shapes and variations in the number of lobes. Notably, plants in Isodose Rings 2, 4, and 5 showed additional symptoms of crinkled leaves. These findings suggest that chronic exposure to gamma radiation can disrupt normal plant development and physiology, leading to visible morphological changes. Changes in morphological characters after gamma radiation have been reported by many researchers in various types of plants including tea (Singh *et al.* 2023), maize (Marcu *et al.* 2013) chrysanthemum (Susila *et al.* 2019), and taro white (Fadli *et al.* 2018). The effect of gamma radiation on morphological character may be attributed to the stimulation of certain physiological processes or genetic responses within the plant (Riviello *et al.* 2022). Besides, chlorophyll mutation which can be seen on leaves, is a good indicator of the response to radiation and might be used as a parameter in accessing the frequencies of mutations at different doses (Azhar & Alsanulkhaliqin, 2014).

Table 2: Morphological changes observed at week 8 after planting

Treatment	Morphological Changes
Control	Normal Color with 5 number of lobes
Ring 1	Chlorophyll degradation Leaf abnormalities (Different shapes of leaves and different numbers of lobes)
Ring 2	Chlorophyll degradation Leaf abnormalities (Different shapes of leaves and different numbers of lobes) Crinkle leaves
Ring 3	Chlorophyll degradation Leaf abnormalities (Different shapes of leaves and different numbers of lobes)
Ring 4	Chlorophyll degradation Leaf abnormalities (Different shapes of leaves and different numbers of lobes) Crinkle leaves
Ring 5	Chlorophyll degradation Leaf abnormalities (Different shapes of leaves and different numbers of lobes) Crinkle leaves
Ring 6	Leaf abnormalities (Different shapes of leaves and different numbers of lobes)

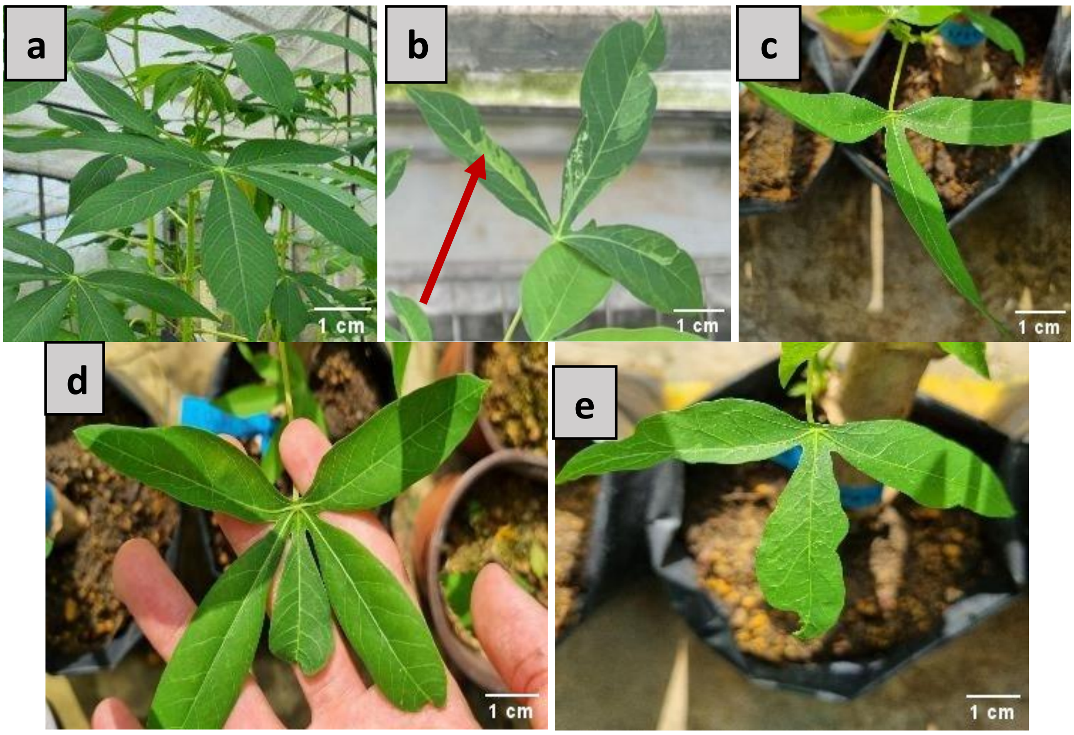

Figure 4: Leaves show (a) Normal color with 5 lobes (Control); (b) chlorophyll degradation; (c) Different numbers of lobes; (d) Different shapes of leaves; and (e) crinkled leaves

CONCLUSION

The study investigated the impact of chronic gamma irradiation on *Ubi Kuning* stem cuttings over 8 weeks, revealing that high doses of gamma rays can significantly alter plant morphology and growth. Survival rates remained consistently high across most treatment groups, indicating a robust tolerance to gamma irradiation. However, plant height and bud formation were adversely affected by higher radiation doses, with the highest cumulative dose (1709.49 Gy) resulting in the lowest plant height and reduced bud counts. Morphological changes, such as chlorophyll degradation and leaf abnormalities, were prevalent in irradiated plants, particularly at higher doses. These findings suggest that while *Ubi Kuning* can withstand substantial gamma radiation exposure, significant doses impede its growth and induce notable morphological mutations. This study can later be improved by observing the growth of the next mutant generation.

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