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Gamma Ray Irradiation Effects on Embryogenic Calli Growth in Indonesian Taro

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ABSTRACT

Colocasia and Xanthosoma are widely distributed in humid tropical areas and primarily found in moderate to high rainfall areas. In Indonesia, *Colocasia esculenta* var. antiquorum (eddoe taro) and *Xanthosoma undipes* (beneng banten taro) are prominent taro. However, traditional vegetative propagation methods often result in limited phenotypic diversity. Gamma irradiation of embryogenic calli presents a promising approach to induce genetic diversity for taro improvement. This study aimed to assess the radiosensitivity of Indonesian taro explants to gamma-ray irradiation by determining the LD_{50} value and evaluating the impact of the gamma ^{60}Co irradiation dose on the proliferation and growth of taro embryogenic calli. The research adopted a completely randomized factorial design, encompassing eight treatments of Gamma ^{60}Co radiation dose ranging from 0 to 27.5 Gy, combined with two treatment media variations on callus formation, with 12 repetitions each. The results showed that gamma-ray irradiation affected callus formation, embryogenic callus proliferation, and the number of globular phases of somatic embryos in both explants. The LD_{50} for *Colocasia esculenta* explants was determined to be 7.23 Gy, while that for *Xanthosoma undipes* explants was 12.84 Gy. These findings underscore the significant effect of gamma-ray irradiation on explants, elucidating its potential to induce mutations and augment genetic diversity in orphan crop species such as Indonesian taro.

Keywords: Araceae, asparagine, browning, callus, Colocasia, malt extract, somatic embryo, Xanthosoma

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INTRODUCTION

The aroids plant family comprises more than 120 genera and 3750 species, and it is widely distributed in humid tropical regions with medium to high rainfall (Vaneker & Slaats, 2012). In addition to their use as ornamental plants, they play a crucial role in horticulture as secondary staples in Asia, the Pacific Islands, and Central South America. Taro is one of the edible aroids found in Indonesia. They serve as staple and functional foods due to their rich starch, fiber, potassium, vitamin C, proteins, glucomannan, and other micronutrients (Cahyanti et al., 2024). Furthermore, taro can thrive in various soil types, including well-drained, dry, and regions with high rainfall (Chaïr et al., 2016; Cahyanti et al., 2022; Chaïr et al., 2016). Some taros in Indonesia are derived from the Alocasia, Colocasia, and Xanthosoma genera.

Colocasia esculenta (L.) Schott var. *antiquorum*, commonly known as eddoe taro, is widely grown in Indonesia owing to its high productivity and delicious tuber taste. The plant has light-green petioles, purple upper parts, and cylindrical tubers (Maretta et al., 2020). Tubers are highly nutritious, containing 3.45% protein, 0.31% fat, 6.07% water, 2.14% ash, 88.03% carbohydrates, and 2.87% fiber (Fidyasari et al., 2017). This taro is also a suitable crop for areas prone to drought as it has good adaptation mechanisms to groundwater fluctuations (Hidayatullah et al., 2020). However, the plant is susceptible to pest attacks, particularly leaf pests, such as grasshoppers (Jayaprakas & Harish, 2022).

The Indigenous beneng banten taro (*X. undipes* K. Koch) is a valuable agricultural commodity in Indonesia, characterized by its large tuber size and high protein and carbohydrate content. It has the potential to be used in various food products and can contribute to food security. The flour contains 82.56% carbohydrates, 3.4% protein, 0.28% and 0.8% fat, and the leaves have been used as a tobacco substitute (Rostianti et al., 2018; Susilawati et al., 2021). As a relatively new domesticated crop, beneng banten taro has yet to undergo significant genetic improvement beyond its wild counterpart (Alghifari et al., 2023).

Despite their nutrient density, historical importance, and wide adaptability, most taro are classified as orphan crops, necessitating genetic improvement. The development of a new cultivar relies on the use of taro germplasm, which demonstrates high genetic diversity and genetic distance. Vegetative propagation in taro leads to a limited range of phenotypic diversity. Prana (2007) noted that traditional crossing methods for breeding *C. esculenta* cultivar take a long time, with plants taking six to eight months to flower after planting. Therefore, enhancing the genetics of taro varieties is not without its challenges, but it is essential for creating new cultivars with promising yields.

An effective strategy for enhancing genetic diversity in taro involves the application of ionizing radiation, such as gamma rays, to induce mutagenesis in embryogenic calli. Gamma-ray irradiation can cause deletions or aberrations in deoxyribonucleic acid

(DNA) owing to its interaction with high-energy electromagnetic radiation. This method is advantageous because embryogenesis originates from individual cells, thus minimizing the formation of chimeric mutants and allowing selection at the cellular level. Gamma rays are important in mutation breeding and in vitro mutagenesis to develop the desired plant traits and increase genetic variability among radiation sources. Gamma-ray mutation induction in embryogenic calli has been applied to enhance temperature resistance in wheat (*Triticum aestivum* L.) and to produce 19 putative mutant plantlets of the Dewata variety, which are believed to be tolerant to high temperatures (Setiawan et al., 2015). Nurilmala et al. (2017) induced somaclonal variation in *C. esculenta* (L.) by exposing shoot explants to gamma irradiation with an LD_{50} of 10 Gy, resulting in up to 51% genetic diversity compared to the parents.

The first step in plant breeding using mutation techniques is to optimize the radiation dose and target radiosensitivity. The radiosensitivity of a plant to gamma-ray irradiation can be evaluated by determining its lethal dose of 50% (LD_{50}). LD_{50} is the radiation that can cause the death of 50% of the exposed plant population. Gamma irradiation has been carried out *in vitro* shoots of cocoyam (*Xanthosoma sagittifolium* L. Schott) with an LD_{50} of 8.7 Gy for the white flesh tuber and 7.6 Gy for the red flesh tuber (Ndzana et al., 2008). Seetohul et al. (2008) showed that 20 Gy of gamma irradiation was lethal to shoot explants of *C. esculenta* (L.). Gamma-ray irradiation doses can positively and negatively affect a given plant. This study aimed to determine the radiosensitivity of two types of taro explants to gamma-ray irradiation and to examine the effect of the irradiation dose on the growth of embryogenic calli.

MATERIALS AND METHODS

Explant Materials and Sample Preparation

The explants used were aseptic plantlets of eddoe taro (*Colocasia esculenta* var. antiquorum) and beneng banten taro (*Xanthosoma undipes* K. Koch) obtained from Tissue Culture Laboratory 3, Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University. The explant was the leaf base of a taro shoot, measuring 10×5×1.5 mm.

Each explant was planted on callus initiation with MS basic media composition (Murashige & Skoog, 1962) enriched with 500 mg L^{-1} malt extract (Merck, Germany), 200 mg L^{-1} l-asparagine (Merck, Germany), 1000 mg L^{-1} cefotaxime antibiotic (Cetaxime, Indonesia), and two levels of plant growth regulator (PGR) addition, TDZ (PhytoTech Labs, USA) and picloram (PhytoTech Labs, USA). Eddoe taro explants were planted on media with the addition of 13.57 µM picloram and 9.04 µM picloram+ 6.81µM TDZ. In comparison, beneng banten taro explants were planted on basic media with PGR added in the form of 4.52 µM picloram+ 6.81µM TDZ and 9.04 µM picloram+ 6.81µM TDZ.

The explants were cultured for three weeks before irradiation to ensure that the taro explants were at the active growth stage for irradiation treatment. Selection was carried out on green and aseptic explants that had uniform growth before being treated with irradiation. Explants were planted in disposable petri dishes measuring 90×90×15 mm, with an explant density of 12 explants per petri dish.

Gamma Ray Irradiation Treatment

Gamma-ray irradiation was performed at the Research and Technology Center for Application of Isotope and Radiation, National Research and Innovation Agency of the Republic of Indonesia, using a ^{60}Co gamma-ray irradiator (Gamma Chamber 4000 Å, Bhabha Atomic Research Centre, India). An irradiation experiment was conducted with ^{60}Co gamma-ray irradiation at 0 (control), 2.5, 5, 7.5, 10, 12.5, 17.5, and 27.5 Gy; the dose rate used was 2.25 Gy/min.

Explant Regeneration

The irradiated explants were then transferred to a new medium with the same composition at a rate of one explant per bottle. Explants were transferred less than 24 h after irradiation to replace the medium that was thought to be toxic to plant cells due to irradiation. Planted explants were stored in a culture room at 23 ± 2°C and a light intensity of 1000 lx. Culture bottles containing explants were maintained by spraying 96% alcohol into the culture bottles every week.

Explant growth was observed every week for eight weeks after irradiation, with the variables being the percentage of explant survival after irradiation, color and condition of the explant after irradiation, percentage of callus, time for callus formation, percentage embryogenic callus, number of somatic embryos (in the globular phase), time for somatic embryo formation, and efficiency of embryogenic callus formation.

This study used a completely randomized design (CRD) with two factors, two combinations of treatment media, and eight combinations of irradiation doses with 12 repetitions in one repetition consisting of one culture bottle with one taro explant; thus, there were 192 observation units for each taro. The data were tested using *F*-test analysis at the α=5% level using Minitab 17 software. The F-test results showing a real effect were further tested using Tukey's range test at the α=5% level. LD_{50} calculations were performed using CurveExpert Professional 2.7.3 software.

RESULTS

Response to Gamma Irradiation

Different gamma-ray doses significantly influenced the formation of calli and somatic embryos (Table 1). Callus formation was observed four weeks after irradiation. Interestingly,

media treatment did not significantly affect callus formation, unlike the irradiation dose, which considerably influenced the percentage of callus formation. The control treatment successfully produced 100% explants with calluses for both taro. In eddoe taro (*C. esculenta* var. antiquorum) explants, an irradiation dose of 17.50 Gy produced a low callus percentage of 20.83%. In contrast, beneng banten taro (*X. undipes*) explants, irradiation doses above 12.50 Gy resulted in low callus percentages of 29.17% (17.50 Gy) and 16.67% (27.50 Gy). Beneng banten taro explants receiving irradiation of 2.50 — 12.50 Gy still formed callus well, with over 85% exhibiting callus. In contrast, this treatment produced a callus percentage of over 33% for eddoe taro.

Table 1
Effect of gamma dose on callus initiation and regeneration in taro

Gamma doses (Gy)	Eddoe taro (*C. esculenta var. antiquorum*)				Beneng banten taro (*X. undipes*)			
	Callus formation[a] (%)	EC induction[b] (%)	Number of $SE^{c}_{(g)}$ ($\bar{x}$)	% EEC[d]	Callus formation[a] (%)	EC induction[b] (%)	Number of $SE^{c}_{(g)}$ ($\bar{x}$)	% EEC[d]
0.00	100.00 a	95.83 a	8.44 a	95.83	100.00 a	100.00 a	24.13 a	100.00
2.50	70.83 ab	50.00 b	4.00 b	66.67	87.50 a	66.67 ab	7.80 b	88.89
5.00	66.67 ab	41.67 b	2.46 bc	71.44	87.50 a	66.67 ab	5.83 b	80.01
7.50	58.33 bc	37.50 bc	1.85 bc	81.82	100.00 a	62.50 abc	6.03 bc	83.33
10.00	70.83 ab	29.17 bc	1.92 c	70.00	95.83 a	58.33 bc	6.00 bc	82.35
12.50	33.33 bc	16.67 bc	2.00 c	57.15	91.67 a	41.67 bcd	3.30 cd	90.92
17.50	20.83 c	16.67 bc	0.88 c	80.03	29.17 b	25.00 cd	1.13 d	100.00
27.50	25.00 c	4.17 c	0.50 c	50.06	16.67 b	8.33 d	0.50 d	66.64
F-Value Gamma doses	**	**	**		**	**	**	
%CV	25.55	27.47	50.55		19.88	25.92	64.07	

Note. (**) has a very significant effect, numbers followed by the same letter in the same column, CV: coefficient of variance, and variables show no significant difference based on Tukey's Studentized Range (HSD) ($p < 0.05$). [a]Callus formation was observed in the fourth week after irradiation. [b]Embryogenic callus induction was observed at the eighth week after irradiation. [c]Number of somatic embryo (globular phase) was observed in the eighth week after irradiation. [d]Efficiency of embryogenic callus formation: %EC induction/% callus formation 100% at the eighth week after irradiation

The induction of embryogenic calli in beneng banten taro and eddoe taro explants was significantly influenced by the level of gamma-ray irradiation. In beneng banten taro explants, a decrease in the ability to induce embryogenic calli below 50% occurred at medium to high doses of irradiation (12.50 — 27.50 Gy), with only 8.33% of embryogenic calli formed at 27.50 Gy. All doses of irradiation on eddoe taro explants reduced the ability of the explants to form embryogenic calli, with a low dose (2.50 Gy) reducing the ability by 50% and a high dose (27.50 Gy) producing only 4.17% embryogenic calli. However,

the efficiency of embryogenic callus induction in both explant types was greater than 50% at all irradiation doses. Even at 17.50 Gy, 100% of the calli produced by beneng banten taro explants induced embryogenic calli in the eighth week after irradiation. The highest irradiation dose (27.50 Gy) for eddoe taro explants reduced the efficiency of embryogenic callus induction by up to 50%.

The dose of gamma irradiation significantly affected the number of somatic embryos produced by taro explants in the globular phase eight weeks after irradiation. The average number of somatic embryos per explant decreased as the gamma irradiation dose increased. The ability of beneng banten taro and eddoe taro explants to produce somatic embryos decreased by 75% compared to that of the non-irradiated treatment. Even though the high-dose irradiation (27.50 Gy) still produced an average number of somatic embryos per explant in all taro explants, it was significantly lower than the low to medium irradiation doses (2.50 — 12.50 Gy) in beneng banten taro and the low dose (2.50 Gy) in eddoe taro.

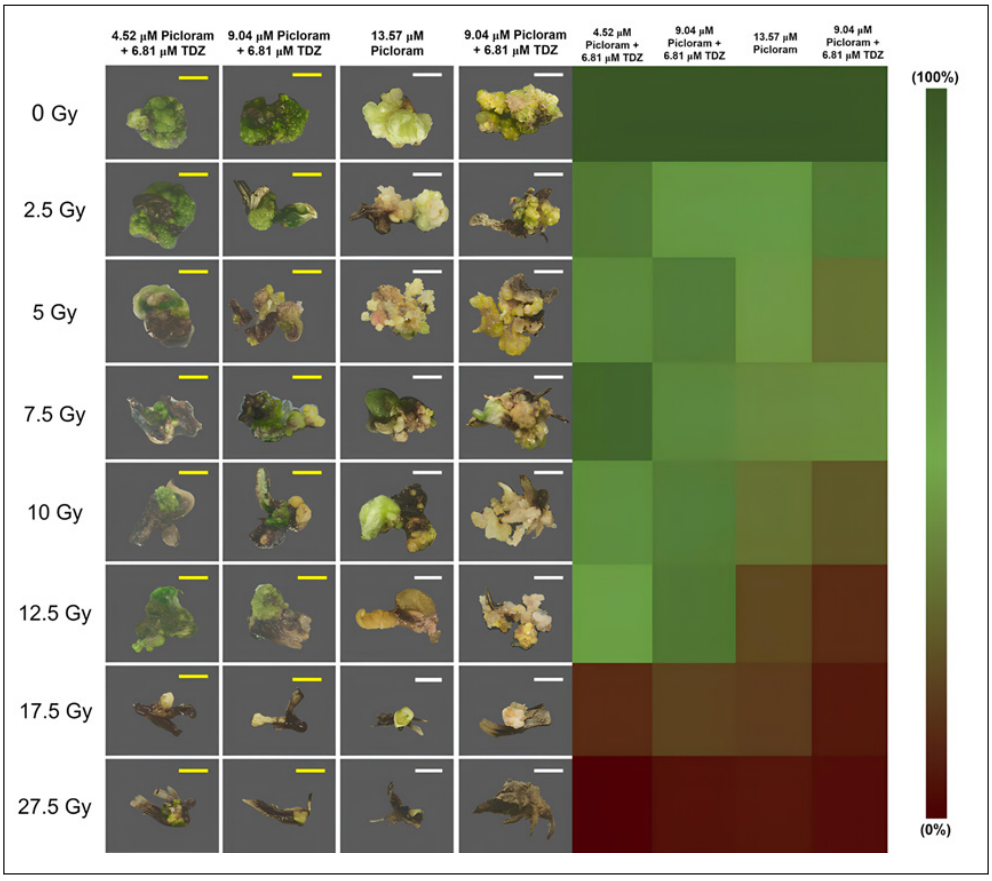

Figure 1. The condition and heatmap of green beneng banten taro (*X. undipes*) and eddoe taro (*C. esculenta* var. antiquorum) explants at eight weeks after irradiation

Note. Yellow bar = Beneng banten taro, White bar = Eddoe taro, Bar = 5 mm

All explant outcomes varied depending on the radiation dose administered after eight weeks of exposure (Figure 1). The control group appeared healthier and greener in color than the other treatment groups. Explants treated with 2.50 — 12.50 Gy of radiation displayed various changes in appearance, such as the callus becoming crumbly or compact, the color of the explant becoming paler, and cell death marked by browning in some parts. In contrast, explants exposed to more than 12.50 Gy of radiation experienced browning and cell death in nearly 80% of samples.

Radio-sensitivity of Taro Explants

The results of this study on the effect of irradiation on taro explant survival are shown in Figure 2. The data revealed that the percentage of explant survival decreased as the irradiation dose increased for both taro. Specifically, gamma irradiation at 12 Gy suppressed the growth of beneng banten taro explants by nearly 50%, whereas a dose of 7.5 Gy suppressed the growth of eddoe taro explants by 50%. However, it is important to note that complete explant death did not occur even at the highest dose level, as 21.59% of beneng banten taro explants and 12.50% of eddoe taro explants remained viable after treatment with 27.50 Gy of irradiation. The differences between control and treated explants were found to be statistically significant ($p \leq 0.05$) for both types of taro, particularly between doses of 12.50 to 27.50 Gy for beneng banten taro and between doses of 7.50 to 27.50 Gy for eddoe taro. Based on these results, the estimated LD_{50} for beneng banten taro explants was 12.84 Gy, whereas the LD_{50} for eddoe taro explants was 7.23 Gy.

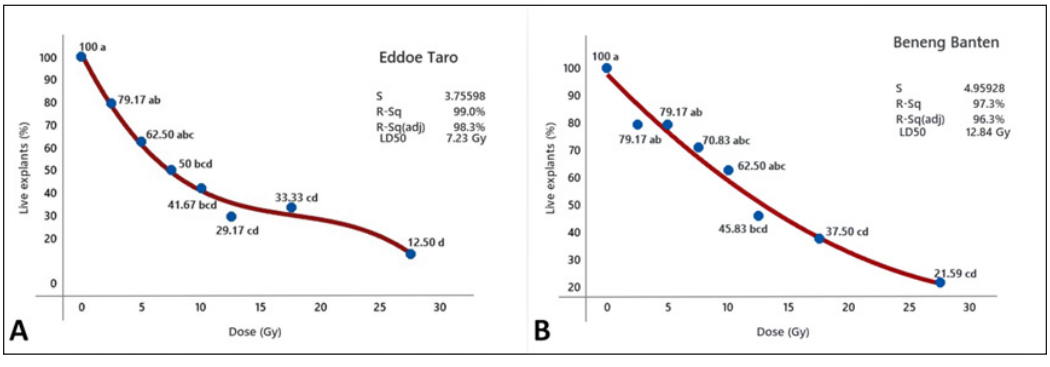

Figure 2. (A) Radiosensitivity curves of eddoe (*C. esculenta* var. antiquorum) and (B) beneng banten (*X. undipes*) taro at eight weeks after irradiation. Numbers followed by the same letter indicate no significant difference based on Tukey's Studentized Range (HSD) ($p < 0.05$)

DISCUSSION

The callus is a type of tissue that forms temporarily through the growth of stem cells. It is an undifferentiated tissue, meaning it has yet to develop into a specific tissue type. Differences

in callus formation may indicate changes in irradiated explants, reflecting their ability to survive radiation-induced cellular damage and resume growth. In this study, low doses of gamma irradiation support callus formation, as demonstrated in *Metroxylon sagu* by adding callus biomass in the 10 Gy gamma irradiation treatment (Riyadi & Sumaryono, 2017). Low doses of gamma rays have a positive effect on the growth of various plants, such as *Hordeum vulgare* L. seeds (Volkova et al., 2019) and shoot formation in bananas (Ali et al., 2020). The positive effect of low doses of gamma irradiation on growth and development may be due to better mobilization of proteins and metabolites and modulation of reactive oxygen species (ROS) levels. There is evidence that ROS could be a crucial molecule that acts as a hub in various phytohormonal cascades connected to changes in phytohormone signaling pathways (Geras'kin et al., 2017; Sewelam et al., 2016).

The influence of gamma irradiation on embryogenic callus induction in explants is thought to be related to the death of explants caused by irradiation, particularly high-dose irradiation. Gamma irradiation at high doses can result in cell disruption and explant death. However, this can also lead to creating more mutant variants through the induction of chromosomal and DNA damage (Agisimanto et al., 2016). Despite this, the relatively high efficiency of embryogenic callus induction in this study suggests that the administered irradiation dose did not affect the callus redifferentiation process into embryogenic calli, which is meristematic and has the potential to develop into somatic embryos. Litz (2004) emphasized the importance of limited proliferation cycles due to irradiation to ensure cell recovery for further development. Similarly, decreased responses to embryogenic callus induction following gamma irradiation have been observed in *Saccharum officinarum* (Hapsoro et al., 2018) and *Agave tequilana* (Valencia-Botín et al., 2020).

Somatic embryos are ideal for mutagenesis because they are derived from a single cell and are thus not susceptible to chimeric development. The decrease in the average number of globular-phase somatic embryos was attributed to a decline in the number of embryogenic calli that could be induced by gamma-ray irradiation. When subjected to high levels, gamma rays produce and accumulate ROS, which damage plant tissues (Liu et al., 2021). Another study found that gamma irradiation resulted in the degradation of the indoleacetic dehydrogenase enzyme, which is crucial for IAA synthesis. Degradation of this enzyme leads to a browning reaction in explants and reduces the regeneration capacity of the plant (Rosmala et al., 2022). Gamma irradiation also decreased the average number of somatic embryos in *M. sagu* (Riyadi & Sumaryono, 2017), *Panax ginseng* (Lee et al., 2019), and *Coffea* spp. (Bado et al., 2021), and *Catharanthus roseus* (Mujib et al., 2022).

Using gamma rays as a mutational agent has benefited plant breeding and germplasm collection. However, high doses of gamma irradiation can hinder the development of living plants, such as *A. tequilana* (Valencia-Botín et al., 2020) and *Philodendron billietiae* (Maikaeo et al., 2024). On the other hand, it can also promote a broader range of genetic diversity, as observed in *Typhonium flagelliforme* (Sianipar et al., 2017), *Etlingera elatior*

(Azzahra et al., 2018), *Vanilla planifolia* (Serrano-Fuentes et al., 2022), and *Zamioculcas zamiifolia* (Beyramizadeh et al., 2023).

Many studies have shown that the exposure of plants to high doses of radiation can harm their growth. It can include hindering germination and causing negative outcomes, such as chromosomal abnormalities, which may eventually result in plant death (Gudkov et al., 2019; Jan et al., 2011; Tan et al., 2023). A common indication of explant death resulting from irradiation is color change. During high-dose irradiation, the explant changes color from green to brown or black due to ROS. As mutagenic agents, gamma rays produce biological effects through the interactions of atoms or molecules in cells, particularly with water. Gamma irradiation can increase the production of reactive ROS, including superoxide anions, hydrogen peroxide, and hydroxyl radicals. This increase in ROS can trigger oxidative stress in various cellular components, resulting in chloroplast deformation, plasmalemma disintegration, and nuclear membrane rupture (Gill & Tuteja, 2010). The accumulation of excess ROS, accompanied by organelle disorganization, leads to cell death, which can be observed in a brownish color (Sharma et al., 2012). The browning and death of calli due to gamma irradiation also occur in *Allium sativum* L. (Maryono, 2020) and *Amorphophallus paeoniifolius* (Rivai et al., 2022).

It is crucial to determine the radiosensitivity of plants before implementing large-scale irradiation processes. Several factors, including planting material, plant genotype, degree of polyploidy, cell development stage, tissue age, and post-irradiation conditions, influence the optimal dosage of gamma rays for mutagenesis. Therefore, conducting radiation sensitivity studies such as LD_{50} determination is essential before incorporating gamma rays into breeding programs (Datta, 2019; Datta, 2023). In vitro, gamma irradiation was conducted on cocoyam shoots (*X. sagittifolium* L. Schott) with an LD_{50} of 8.7 Gy for white-fleshed tubers and 7.6 Gy for red-fleshed tubers (Ndzana et al., 2008). The effective dose (LD_{30}) in cultures of taro shoots (*C. esculenta* (L.)) was found to be 7.65 Gy, causing a 30% reduction in growth. Furthermore, 20 Gy gamma irradiation is lethal to taro shoot explants (Seetohul et al., 2008).

The present study produced a higher LD_{50} than previous research on taro families that utilized irradiation despite employing shoot base explants as the planting material. This difference is attributable to adding malt extract and l-asparagine to the media, which serve as additional carbohydrates and amino acid sources. Malt extract contains approximately 90% carbohydrates, amino acids, and phenolic compounds, such as ferulic acid (Chaves et al., 2019). L-asparagine is an adaptable amino acid that participates in several biological processes, such as plant growth and adjustment to environmental stressors (Han et al., 2022). In addition to inducing somatic embryos, these two components are thought to facilitate cell adaptation, particularly in cell walls that experience oxidative stress due to the induction of gamma rays. Numerous studies have shown that plant cells possess several mechanisms for coping with oxidative stress. These mechanisms include activating

and synthesizing antioxidants, enzymes, and non-enzymatic substances. Some of these substances can reduce the catalytic activity of transition metals, such as polysaccharides in cell walls and structural proteins (Gill & Tuteja, 2010; Hassinen et al., 2011; Zagorchev et al., 2013). Consequently, gamma irradiation at the highest dose (27.5 Gy) did not result in the complete death of the explants.

CONCLUSION

In this study, gamma irradiation affected the initiation and proliferation of embryogenic calli in eddoe and beneng banten taro plants. Specifically, the LD_{50} for eddoe taro explants was 7.23 Gy, while the LD_{50} for beneng banten taro explants was 12.84 Gy. Additional studies are necessary to investigate the effects of adding malt extract and l-asparagine on the survival of explants. The results showed that lower doses of gamma irradiation can increase explants' ability to form calluses, whereas higher doses can decrease their capacity to redifferentiate into globular-phase somatic embryos. Using gamma rays for mutation induction is a potential method for increasing genetic diversity or improving orphan crop plant species, such as Indonesian taro.

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