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Optimal Dose Determination of Gamma Ray Irradiation of *Alocasia suhirmaniana* Yuzammi & A. Hay

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ABSTRACT

Alocasia suhirmaniana Yuzammi & A. Hay belongs to the Araceae family and has the potential for ornamental use. Nevertheless, this plant exhibits a limited leaf count and pliable leaf stems, necessitating the application of breeding techniques for its improvement. One important strategy empeloyed to improve the quality of ornamental plants is the breeding, which involves the use of gamma ray irradiation techniques. The objective of this study is to determine the developmental patterns and appropriate dosage levels of LD20 and LD50 Gamma ray irradiation in *A. suhirmaniana* callus. The development of callus, the quantity of shoots, and the establishment of rooted explants exhibit a stochastic pattern, with a decrease observed at a dosage of 5 Gy and an increase observed at 10 Gy. The LD20 and LD50 values for Gamma ray irradiation in *A. suhirmaniana* callus fall within the dose range of 7.14 Gy and 15 Gy, respectively. This dosage range is recommendedto achieve a greater diversity in genotypes and phenotypes. By successfully finding the optimal dosage of gamma ray irradiation, plant selection can be improved through enhancing genotype and phenotype characteristics.

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INTRODUCTION

Alocasia species, belonging to the Araceae family, are commonly known as taro and are predominantly distributed in Indonesia. According to Asih et al. (2022), the distribution of *Alocasia* species is observed across various regions including Sumatra, Java, Kalimantan, Sulawesi, Maluku, and Papua. The species under consideration predominantly inhabits lowland tropical plants, specifically those that thrive in regions characterized by high levels of humidity. *Alocasia* species are primarily found in specific regions and are not widely distributed, except in cases where human activity has influenced their range (Hay, 1998). *Alocasia* is primarily utilized as a ornamental leaf plant, although it also serves various other purposes such as food, feed, and medicine (Yuzammi, 2018).

Alocasia suhirmaniana Yuzammi & A. Hay, a species belonging to the *Longiloba* group, is known to be endemic to Sulawesi, as documented by Hay in 1998. The species under investigation was discovered in the lowland areas of secondary forests located in the Kolaka and North Kolaka regencies of Southeast Sulawesi Province (GBIF, 2023). The species is a terrestrial plant with a height ranging from 50 to 65 cm. It possesses 1 to 3 strands of leaves, characterized by a yellowish green petiole and tilted brownish spots. The leaf strands are peltate, measuring approximately 55 cm in length. These strands are are independent and exhibit a slightly undulated margin. The color of the leaves remains undetermined. According to Yuzammi

(2018), the leaf exhibits a dark green gloss on its upper surface, which is further accentuated by the presence of light green leaves. in contrast, the lower surface of the leaf is characterized by a dark purple coloration.

The species under investigation exhibits a noteworthy characteristic due to its unique combination of vibrant coloration and aesthetically pleasing leaf morphology. Nonetheless, the cultivation of this particular species as an ornamental leaf plant has not gained significant popularity due to its inherent limitations. One of the primary factors contributing to its lack of appeal is the relatively low leaf count, coupled with the presence of broad leaves and diminutive leaf stalks, which collectively detract from its aesthetic value. The breeding of *A. suhirmaniana* can be undertaken to enhance its efficacy and contribute to the conservation of this species. The utilization of gamma irradiation for inducing genetic variations is considered a potential breeding technique in plants.

Gamma ray radiation is a form of electromagnetic radiation generated by radioisotopes and nuclear reactors. It possesses high energy and is capable of penetrating through various materials. Examples of sources that emit gamma rays include Cobalt-60 (60Co) and Cesium-137 (137Cs) (Dwimahyani & Widiarsih, 2018). Gamma ray radiation is known to possess high lethality when administered at high doses. However, when administered in controlled doses, gamma ray radiation exhibits significant utility in a wide range of applications. According to Dowlath et al. (2021), radiation has the potential to induce genotoxicity, which refers to its ability to cause damage to genetic material. Additionally, radiation can lead to mutations that can impact heredity. The phenomenon of low dose radiation is characterized by a reduced occurrence of side effects, which can be attributed to the activation of defense mechanisms in plants when exposed to low doses of gamma rays. These defense mechanisms play a crucial role in mitigating and overcoming potential damage to the plant. It has been observed that exposure to high levels of irradiation can affect the phenotypes of plants including changes in various cell organelles and chemical components (Kriston et al., 2011; Ali et al., 2015). The observed phenomenon has the potential to result in detrimental effects on the DNA structure, leading to cell apoptosis. However, it is worth noting that it can also lead to the introduction of genetic variation. One crucial aspect to consider is the identification of the optimal dosage level to prevent lethality and promote enhanced genetic diversity within an individual.

The application of gamma ray irradiation has been extensively employed in plant research to induce genetic diversity. Several studies have been conducted on the *Dendrobium discolor* Lindl. species. One such study examined the lethal doses (LD20 and LD50) of irradiation, which were found to be 22.16 Gy and 58.8 Gy, respectively. Additionally, the irradiation resulted in the development of a rosette-like structure in the leaves (Handini & Aprilianti, 2020). The study conducted by Toogatorop et al. (2016) involved subjecting *Coleus Blumei* to gamma ray irradiation at varying doses ranging from 0 to 27.5+27.5 Gy. This treatment resulted in noticeable variations in both the color and pattern of the leaves. The objective of this study is to determine the developmental patterns and appropriate dosage levels of LD20 and LD50 Gamma ray irradiation in A. suhirmaniana callus.

MATERIALS AND METHODS

The study was conducted in the Laboratory of Culture Center of Research Center for Applied Botani – National Research and Innovation Agency, which serves as the primary research facility for investigating botanical phenomena and conducting experiments. The material utilized for irradiation consists of *A. suhirmaniana's* callus plantlets, cultivated in MS (Murashige and Skoog) media supplemented with 0.5 mg/L Tdz (Thidiazuron). The callus plantletshas been cultivated for a duration of 12 weeks after planting (referred to as 12mst) and were designated as the initial vegetative mutant (MV0). The specimens were subjected to gamma ray irradiation using cobalt 60 (60 Co) as the source. The doses administered were 0, 5, 10, 15, and 20 Gray (Gy). Subsequently, the explant was transferredto the Murashige and Skoog (MS) media supplemented with 0.5 mg/l of NAA (1-Naphtaleine acetic acid), referred to as MV1. The radiocences were observed by assessing the LD50 (Lethal Dose 50) and LD20, which represent the doses of irradiation that result in a 50 percent and 20 percent reduction in viability, respectively.

The experimental design employed in this study was a completely randomized design (CRD) with a single treatment factor, specifically the dose of irradiation. The experimental setup involves administering a dosage comprising of 5 callus explants, with each dosage being replicated 3 times. Observations were conducted 12 weeks post-treatment (MSP) to evaluate various parameters including diameter of callus, percentage of viable explants, number of shoots per explant, percentage of rooted explants, and average number of roots. The determination of the number of roots was achieved through the utilization of an index accompanied by a corresponding description. Specifically, the symbol (-) denotes the absence of root growth, while the symbol (+) signifies the presence of fewer than ten roots. Moreover, the symbol (++) was employed to indicate the existence of a root count ranging from ten to thirty. The symbol (+++) represented a range of 30-50 roots, while the symbol (++++) indicated a quantity of 50 or more roots.

RESULTS AND DISCUSSION

The findings of this investigation revealed a noticeable decline in the growth of callus in *A. suhirmaniana* as the dosage of gamma ray irradiation increased. The growth response observed in this study refers to the percentage of live, sprout, and rooted individuals, as depicted in Figure 1. It has been observed that an elevated dosage of gamma ray irradiation can lead to a heightened level of damage to plant cells, potentially resulting in cellular death. Gamma rays possess the ability to deeply penetrate tissue due to their high energy levels, surpassing those of protons and X-rays. Consequently, they pose a potential risk by causing cellular damage (Amano, 2006).

Based on the observations, it can be inferred that the response of root growth is significantly high in almost all living explants. The phenomenon of root growth percentage can be influenced by various factors, among which the culture medium factor holds a significant role. It has been observed that the inclusion of ZPT NAA in the growth medium has a stimulating effect on root development compared to shoot development. Consequently, the percentage increase in root growth is higher than that of shoot growth. According to Thao et al. (2003), when using MS media supplemented with ZPT (Growth Regulatory Substance) NAA at a concentration of 0.5 μm, it was observed that callus of *A. micholitziana* Sander exhibited better root induction compared to shoot induction. Meanwhile, our research findings indicate that the most effective treatment for promoting shoot growth is the application of ZPT BA (Benzylaminopurin) at a concentration of 0.5 μm. This particular treatment has demonstrated the highest percentage of shoot growth compared to other treatments evaluated in our study. Then, our research shows that increasing the dose of gamma ray irradiation causes a decrease in the growth and development of *A. suhirmaniana.*

Gamma irradiation is a valuable tool for inducing mutations in ornamental plants, including Alocasia species, to enhance genetic variability and develop new cultivars (Saika Anne & J. Lim, 2020). The optimal irradiation dose is crucial and species-dependent, affecting plant growth, development, and mutation induction (Aros et al., 2012). Studies on related plants, such as Aglaonema and taro (*Colocasia esculenta* (L.) Schott) have shown that gamma irradiation can decrease plant viability, alter leaf characteristics, and change color patterns (Ritonga & Sukma, 2017; Nurilmala, 2017). Lower doses can induce beneficial mutations, with 10 Gy showing the highest impact on leaf number in taro (Nurilmala, 2017). These findings provide insights for determining appropriate gamma irradiation doses for *A. suhirmaniana* breeding programs.

LD_{20} dan LD_{50} on *Alocasia suhirmaniana* callus

The findings from the analysis indicate that the administration of gamma rays within the range of 0-20 Gy can result in fatal outcomes, which align with the observed increase in dosage by up to 26.6%. According to the results obtained from the analysis utilizing Curve Fit, the equation representing the Quadratic Fit line is y = 99.7-2.34x-0.06x2. The degree of accuracy, denoted as R, was determined to be 0.999. These results are illustrated in Figure 2. The equation reveals that the LD20 and LD50 values were determined to be 7.11 and 15.19 Gy, respectively. The obtained LD value suggests that callus explants were utilized in the study. Our observations indicate that *A. suhirmaniana* demonstrates a significant susceptibility to gamma ray irradiation, highlighting the need for further investigation into its biological responses and potential implications. As indicated by Handini & Aprilianti (2020), the initial stage in the identification of stable mutant candidates involves the determination of LD20 and LD50 values. The dose range from LD20 to LD50 has been observed to induce alterations in phenotypes related to certain plant characteristics, specifically leaf morphology.

The influence of plant type and material on cell sensitivity to a specific dosage is of significant importance. The utilization of callus explants has been widely acknowledged as a highly effective method for gamma ray irradiation due to its suitability for testing with relatively low doses.

Additionally, the canal structure of callus explants is relatively simple, resulting in a higher percentage of genetic stability. Several studies have been conducted using various plant materials to investigate the potential of callus utilization at reduced doses of gamma ray irradiation. According to Padate et al. (2008), the LD50 value observed in the *Saccharum officinarum* L. callus is 20 Gy. The LD50 values obtained from research conducted on *Nepenthes ampullaria* Jack plant explants exhibit variations between the NAM and NAM Riau variants. Specifically, the LD50 value for the NAM variant is 31.01 Gray, whereas the LD50 value for the NAM Riau variant is 41.57 Gy (Isnaini and Novitasari, 2020). The LD50 value of the *D. discolor* protocrome, as reported by Handini and Aprilianti in 2020, is relatively high at 58.8 Gy.

Radiosensitivity refers to the degree of sensitivity of a material to radiation doses, which requires careful analysis to determine the optimal doses, also known as LD50. The LD50, or median lethal dose, is considered an optimal dosage due to its ability to induce a significant reduction in plant viability, up to 50%. Consequently, the likelihood of DNA alterations occurring is greatly increased. However, it should be noted that the occurrence of mutation is primarily random. This means that as more plants survive, the likelihood of mutation increases. As a result, some studies also employ LD20 to investigate this phenomenon. The utilization of LD20 is widely acknowledged to have a negligible impact on DNA, resulting in a mere 20% cell mortality. However, it is worth noting that certain properties may undergo alterations as a consequence of the selection process aimed at achieving desired characteristics (Maluszynski et al., 2009; Shu et al., 2012).

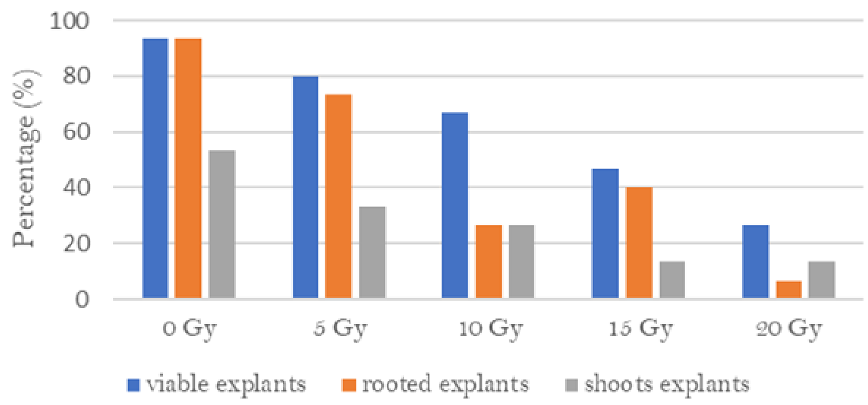

Figure 1. *Alocasia suhirmaniana* callus growth response to the irradiation of gamma rays

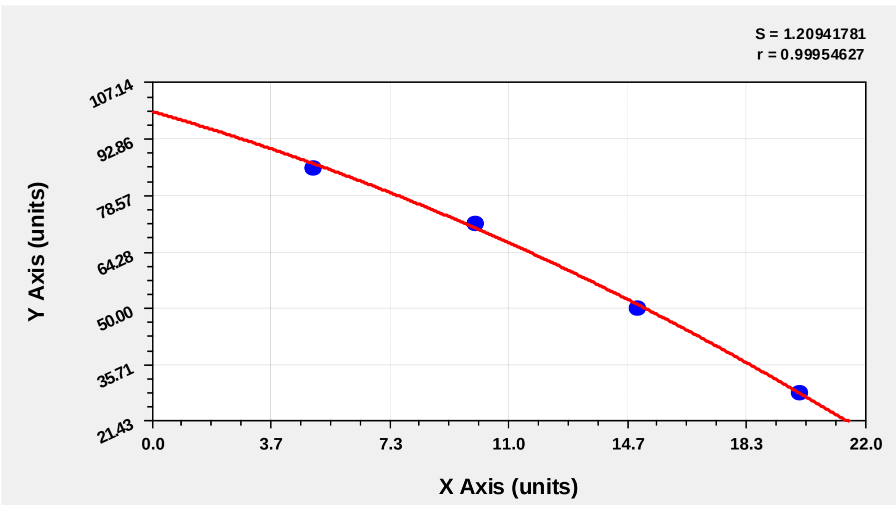

Figure 2. The Radiation Calus Percentage Curve Radiation Results After 12 MSP in *Alocasia suhirmaniana*

Plant response after gamma ray irradiation

The data collected during the study revealed a notable disparity in the diameter of the callus and the quantity of roots generated. The diameter of the callus in the control plant reaches a maximum of 3.21 cm, whereas at a dose of 20 Gy, it only reaches 2.08 cm. Similar observations were also observed in the response to reduced root growth. The response may potentially be attributed to the effect of gamma ray irradiation. As stated by Chaudhuri (2002) in Ali et al., (2015), it the application of gamma ray irradiation has the potential to decrease both root and shoot growth. Nevertheless, the experimental application of gamma ray irradiation did not exhibit a significant impact on the quantity of shoots, as indicated in Table 1. The growth of callus, shoots, and roots showed a decline in response to doses of 5 and 10 Gy. I Intriguingly, there was a noticeable upward trend in growth at a dose of 15 Gy. This phenomenon can potentially be attributed to the random nature of physical mutations.

The impact of gamma ray irradiation extends beyond the mere impairment of cell organelles, as it also influences the characteristics of plant phenotypes. Dwimahyani & Widiarsih (2018) suggested that in order to obtain a genetically stable mutant plant, election must be conducted for a minimum of four to five generations. Further qualitative character selection is required to obtain stable plants through subculture (Figure 3). A study conducted by Aisyah et al. (2009) examined the impact of gamma ray treatment on quantitative characters. The results of their research indicated that the treatment of gamma rays did not have a statistically significant effect on these traits. The mutant arose as a result of alterations in qualitative traits, specifically the color, pattern, and shape of the flower petals. These phenotypic changes became evident starting from the second generation.

Gamma ray irradiation affects plant growth and development in vitro, with higher doses generally leading to decreased shoot and root formation. Low-dose gamma radiation can stimulate growth in plant tissue cultures, while higher doses inhibit development. In *Dracaena surculosa* Lindl., 10 Gy irradiation produced the highest number of shoots, leaves, and roots, as well as increased leaf thickness and vascular bundle size (Sakr et al, 2013). Similarly, 10 Gy irradiation resulted in the highest leaf count for Bogor taro (*C. esculenta*), though 20 Gy proved lethal (F. Nurilmala, 2017). In *Amorphophallus paeoniifolius* (Dennst.) Nicolson, doses above 15 Gy significantly reduced callus survival, with LD20 and LD50 at 1.75 and 12.84 Gy, respectively (Rivai et al., 2022). Similarly, in *Gerbera jamesonii* Adlam, 20 Gy irradiation reduced shoot regeneration and callus fresh weight (Hasbullah et al., 2012). In *D. discolor*, LD20 and LD50 were determined at 22.16 and 58.8 Gy, respectively, with 15-30 Gy doses inducing morphological changes in leaves (Handini & Aprilianti, 2020). These studies suggest that effective mutation induction occurs between LD20 and LD50, with species-specific optimal doses for desired effects on shoot and root formation.

Table 1. Interaction of growth variations to irradiation doses in the callus of *Alocasia suhirmaniana*

Dosage (Gy)	Callus diameter (cm)	Σ shoot per explan	Σ roots per explan
0	**3.21a**	**4.64a**	**+++ (a)**
5	3.13a	1.17a	++ (a)
10	2.09b	2.00a	+ (b)
15	2.57ab	1.00a	++ (a)
20	2.08b	0.50a	+ (b)
F-test	***	ns	***

Note: All data uses 5 explants with 3 replications, NS is not significant, *= p <0.1, ** = p <0.05, *** = p <0.05 analyzed using further test anova DMRT

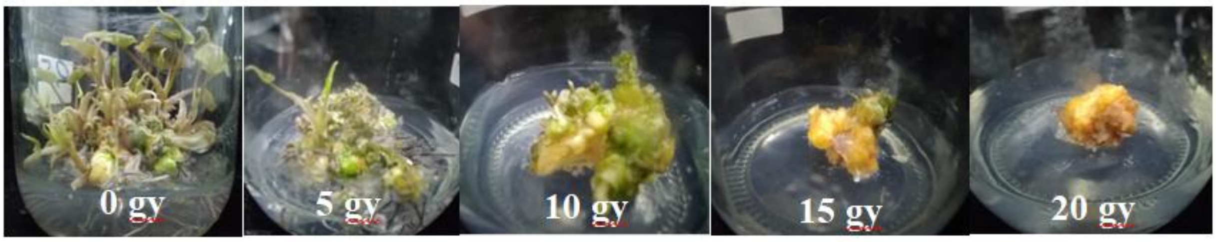

Figure 3. *Alocasia suhirmaniana* callus generation MV1 after irradiation treatment

Gamma ray irradiation can significantly affect in vitro plant growth and development. The number of shoots at a dose of 10 Gy is higher than 5 Gy, which is assumed to occur due to random mutations. (Table 1.) Mutations can lead to an increase in metabolism. Gamma radiation offers a more widespread approach for enhancing metabolite production across various plant parts and in vitro cultures (Khalifa et al., 2022). The optimal dose of gamma irradiation is crucial for achieving the desired mutations and metabolite accumulation, with effects varying depending on the plant species and tissue type (Murthy et al., 2024; Shukla et al., 2021).

CONCLUSION

The findings of this study lead to the conclusion that the LD20 and LD50 values fall within the range of 7.11 and 15.19 Gray. It has been observed that exposure to gamma ray irradiation has a significant impact on the reduction of root growth and shoots. This effect is directly correlated with the increase in the dosage of gamma rays. The administration of gamma ray irradiation has also been observed to impact both the diameter of callus growth and the quantity of shoots generated. The utilization of callus explants has been found to be highly effective in various applications. Additionally, it has been observed that the treatment can induce the development of more stable cells. Remarkably, this effect can be achieved using a relatively low dosage of gamma rays.

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