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Effects of gamma irradiation on morphological changes in Porang (*Amorphophallus muelleri* Blume)

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Abstract. Porang is agricultural plant that become important in the recent years. However, harnessing them through traditional genetic breeding is time-consuming and expensive. Inducing mutagenesis may be a short-time option for its genetic improvement. Therefore, the aim of this reserach is to determine the effect of gamma irradiation in the morphological changes of Porang. This research used completely randomized design (CRD) consisted of 6 levels of radiation including 0 Gray, 2 Gray, 4 Gray, 6 Gray, 8 Gray and 10 Gray. All treatments were replicated 4 times to account for statistical variability. The 30 days after planting of explants were then exposed to varying doses of gamma irradiation using a gamma cobalt-60 at Badan Tenaga Nuklir Nasional (BATAN) Jakarta Indonesia. Porang height, total shoot and leaves, width and length of leaves were characterized after irradiation. Quantitative data were analyzed by using Anova one way and duncan on 5% significance level. Level irradiation of 2 Gray affected the number of shoots and leaves, plant height, width and length of leaves and leaf morphology and colour. Gamma radiation has succeeded in increasing the agricultural value of porang and producing porang with higher genetic diversity than wildtypes.

1. Introduction

Porang (*Amorphophallus muelleri*) is a species of perennial flowering plant belonging to the genus *Amorphophallus* [1]. Porang is native to Indonesia, particularly found in regions like Java, Sumatra, and Borneo [2]. Porang is known for its underground tuber, which is the part of the plant that is primarily used and harvested. The tuber of the Porang is rich in glucomannan [3], a type of water-soluble dietary fiber [4], that often used in various food products [4], particularly as a thickening agent [6] or a dietary supplement [7] due to its ability to absorb water and create a gel-like substance. With increasing demand for alternative food sources and sustainable crop production [8], Porang has garnered renewed attention as a potential candidate for cultivation and exploitation [2]. However, challenges such as long growth cycles, susceptibility to diseases [9], and low genetic diversity have hindered its full-scale adoption and improvement.

Gamma irradiation presents an innovative approach to overcoming these challenges by inducing mutations [10] that could lead to desirable traits such as increased tuber yield [11], enhanced disease resistance [12], and improved adaptability to varying environmental conditions. While studies on the effects of gamma irradiation have been conducted on various crops [10], the specific impact on Porang remains relatively underexplored. Understanding how gamma irradiation influences the morphological characteristics of Porang plants is crucial for harnessing its potential in crop improvement programs.

Gamma irradiation, a powerful ionizing radiation source [14], has found extensive applications in various fields, including agriculture [12] and plant science [15]. In recent years, the exploration of its impact on the growth, development, and physiological responses [16] of plants has gained significant attention due to its potential for inducing genetic variability [17] and aiding in the development of improved plant varieties [18]. Therefore, the aim of this research is to investigate the effects of gamma irradiation on morphological changes of Porang (*Amorphophallus muelleri* Blume). By examining changes in plant growth, leaf structure, and other relevant traits, valuable insights can be gained into the potential benefits and risks associated with gamma irradiation as a tool for Porang improvement. Additionally, understanding the underlying physiological and genetic mechanisms behind these changes can contribute to the broader understanding of plant responses to ionizing radiation and aid in the development of strategies for sustainable crop enhancement.

2. Methods

2.1. Experimental design

A completely randomized design (CRD) was employed to ensure unbiased sampling and replication. This research used 6 different gamma radiation doses including the control, 2 Gy, 4 Gy, 6 Gy, 8 Gy and 10 Gy. All treatments were replicated 4 times to account for statistical variability. The experimental setup was maintained in a controlled environment with consistent temperature, humidity, and light conditions.

2.2. Plant material and irradiation

Healthy and uniform Porang (*Amorphophallus* muelleri Blume) explant were obtained from previous research on Plant Tissue Culture Laboratory, Biology Department Universitas Islam Negeri Maulana Malik Ibrahim Malang. The explants were selected based on similar size and appearance. Prior to irradiation, the explants were subculture in chamber to eliminate any microbial contamination. The 30 days after planting of explants were then exposed to varying doses of gamma irradiation using a gamma cobalt-60 at Badan Tenaga Nuklir Nasional (BATAN) Jakarta Indonesia. The doses of irradiation ranged from 2 Gy to 10 Gy, with a control group of non-irradiated explant. After irradiation the explants was transferred to an MS medium supplemented with 2.2 µM of 6-benzylaminopurine (BAP). All cultures were preserved at 25±2ºC under 24-hour white illumination (1,500 lux) for 50 days. After 50 days in MS medium, plantlets were ready for analysis.

2.3. Data collection

A quantitative and qualitative of morphological character were measured after irradiation. A quantitative character including plant height, number of shoots, and leaf wide and length were measured at plantlets 50 days after planting. A qualitative character including colour and character of leaf changes after irradiation were also observed. All morphological changes were documented using digital imaging techniques.

2.4. Data analysis

Statistical analysis was performed using SPSS 16.0. Data obtained from morphological measurements were subjected to analysis of variance (ANOVA) to determine significant differences among treatments. Duncan tests were conducted to identify specific differences between individual treatment groups. Qualitative data was analysed descriptively by comparing between control and treatment.

3. Result and discussion

3.1. Morphological changes after gamma irradiation

Different doses of gamma radiation have a significant effect on the morphological characters of Porang (Table 1). A gamma radiation dose of 4 Gy had the highest plant height and leaf length and width compared to other treatments whereas dose of 2 Gy has the highest total shoot and root (Table 1) (Figure 1). Porang exposed to gamma radiation above 4 Gy has decreased plant height and leaf length and width whereas radiation dose above 2 Gy decrease total shoot and root (Table 1).

Table 1. Effect of gamma rays on porang morphology

Radiation doses	Plant high	Leaf length	Leaf wide	Total shoot	Total root
0 Gy	2,4958^{c}	1,0625^{b}	0,7283^{c}	3,6667^{b}	5,6667^{bc}
2 Gy	3,0046^{cd}	1,2383^{bc}	0,8075^{c}	**5,5842^{c}**	**8,7492^{d}**
4 Gy	**3,5000^{d}**	**1,4367^{c}**	**0,8529^{c}**	4,5008^{bc}	6,5000^{c}
6 Gy	2,2167^{bc}	1,0400^{b}	0,6367^{bc}	4,6667^{bc}	3,7508^{b}
8 Gy	1,4083^{ab}	0,6708^{a}	0,4142^{ab}	4,3333^{bc}	0,5833^{a}
10 Gy	0,6833^{a}	0,5250^{a}	0,3450^{ab}	0,5833^{a}	0,2492^{a}

Changes in morphological characters after gamma radiation has been reported by many researchers in various types of plants including tea [19], maize [16], chrysanthemum [21] and taro white [20] However, the radiation dose to change morphological characters is different in each plant such 8-10 Gy in tea plant, 10 Gy in chrysanthemum and 2-4 Gy in this research. The radiosensitivity differences of various plant material may the reason why every plant even varieties have their own optimum radiation dose [22].

The effect of gamma radiation on morphological character may be attributed to the stimulation of certain physiological processes or genetic responses [10] within the plant, resulting in enhanced growth. The ability of gamma ray to ionize atoms in plant tissue which ultimately produce reactive oxygen species (ROS) [21], triggers the oxidative stress response to produce various defense enzymes and antioxidant [10]. Finally, it becomes evident that radiation doses can be tailored to promote specific desirable traits in Porang, offering a novel approach to selective breeding and genetic modification.

Gamma radiation, as a tool in plant science, presents both potential benefits and risks, too high radiation dose produce death plant and to low radiation dose does not produce changes in morphological characters [10]. Therefore, research on optimization of radiation dose in each plant is absolutely needed. This study's findings also illuminate the potential hazards associated with excessive radiation exposure. Radiation doses exceeding 4 Gy were found to have adverse effects on Porang, including reduced plant height, smaller leaves, and diminished overall leaf and shoot numbers (Figure 1 and 2). This adverse impact underscores the need for caution when applying gamma radiation in agricultural practices [31]. The delicate balance between stimulating desired traits and avoiding detrimental consequences becomes evident, underscoring the importance of precision in radiation dosage selection.

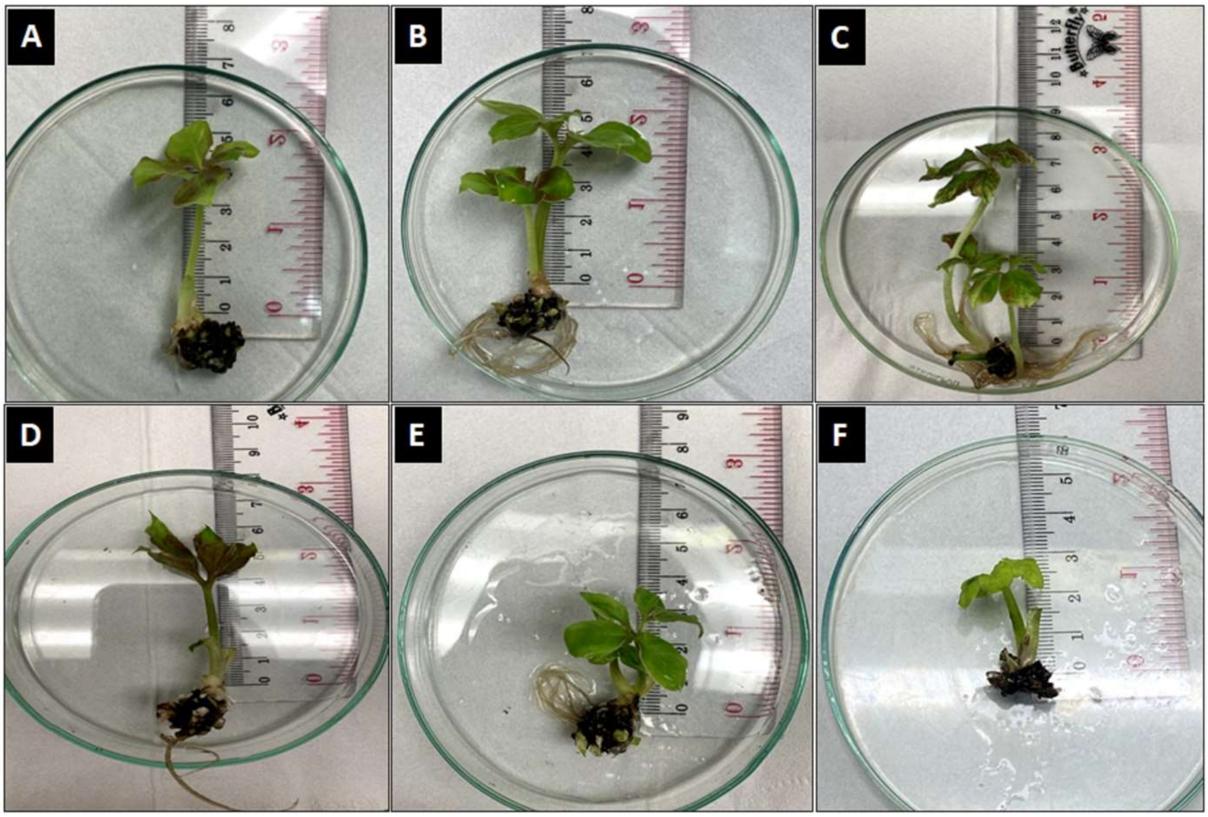

Figure 1. Porang height on 50 days after gamma radiation. A: 0 Gy, b: 2 Gy, c: 4 Gy, d: 6 Gy, e: 8 Gy, dan f: 10 Gy.

The findings of this research also underscore the necessity for further research to elucidate the underlying mechanisms through which gamma radiation influences plant morphology. Detection of antioxidant-related gene and expression of DNA-Repair-Related genes by using RT-PCR [23] or detection of secondary metabolite pathway [24] perhaps a way to reveal the facts behind morphological changes after gamma radiation. Understanding the physiological and genetic pathways involved in the plant's response to radiation could potentially enable scientists to fine-tune radiation exposure for precise control over plant characteristics [32].

Detection of gene expression related to glucomannan synthesis may be the most important thing to know after gamma irradiation [34] since glucomannan is the main component of Porang tubers which makes this plant economically valuable [2]. Understanding the regulatory mechanisms of these genes and their expression can lead to increased glucomannan production, which can have various applications, including improving plant strength and resilience [33]. Further research in this area is essential to unlock the full potential of manipulating gene expression for glucomannan synthesis.

Detection of other gene expressions that are also important to know after gamma radiation are genes related to calcium oxalate crystal synthesis. Calcium oxalate is a plant metabolite that is often found in porang tubers [35]. The high content of calcium oxalate in porang tubers is an obstacle to the management of porang tubers. Therefore, the silencing of related gene of calcium oxalate synthesis is the main goal in sustainability of porang cultivation.

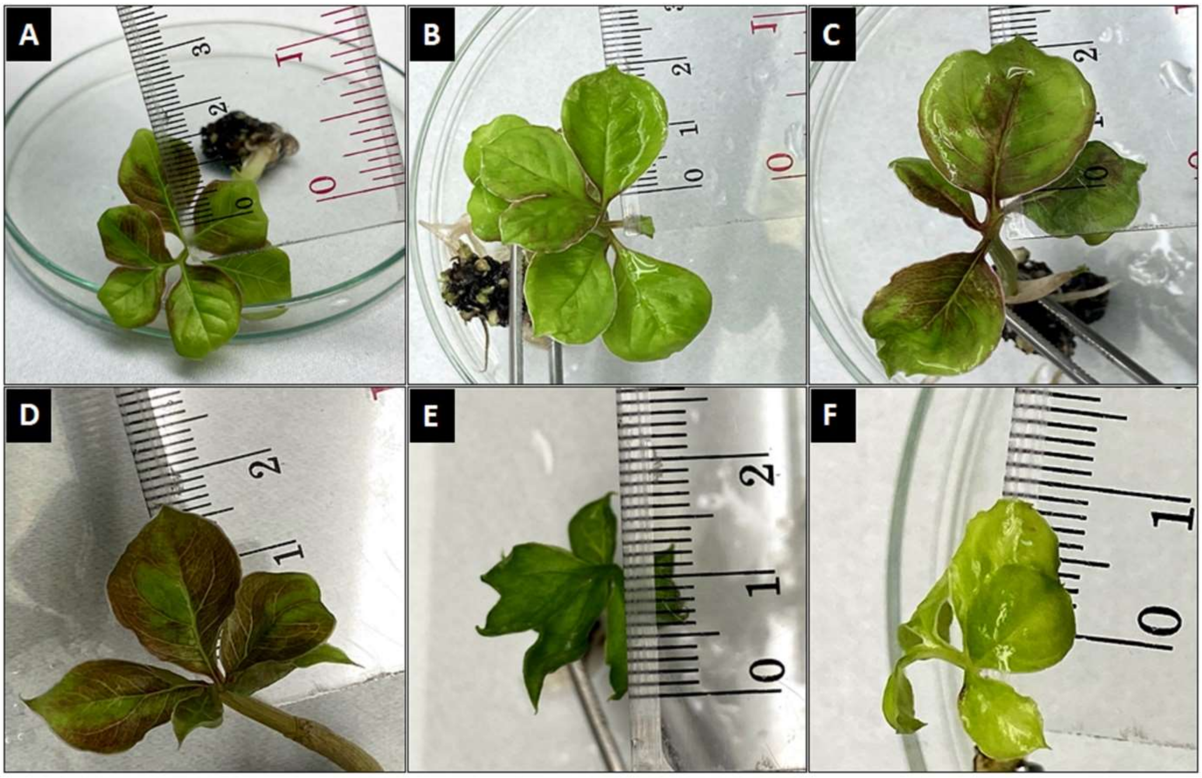

Figure 2. Length and width of Porang leaves on 50 days after gamma radiation. A: 0 Gy, b: 2 Gy, c: 4 Gy, d: 6 Gy, e: 8 Gy, dan f: 10 Gy.

3.2. Leaf chlorophyl mutation

Various levels of gamma radiation exposure resulted in a range of mutations within the leaf chlorophyll (Figure 3). Gamma radiation at dose 2 and 4 Gy resulted chlorophyll mutant white and read streak indicating a potential genetic response to radiation. Furthermore, at a higher dose of 6 Gy, the plants exhibited a dwarf porang phenotype (Figure 3), suggesting severe damage to cellular processes and growth regulation.

Gamma radiation can induce DNA damage and mutations, which may result in altered plant phenotypes [30]. This outcome aligns with previous research on the dose-dependent effects of gamma radiation on plant biology. Gamma radiation exposure has been observed to induce chlorophyll mutations characterized by white and red streaks in various plant species, which provides evidence of a potential genetic response to radiation [25][26]. This phenomenon highlights the impact of gamma radiation on plant genetics and the resulting morphological changes [27]. Additionally, studies have explored the effects of gamma irradiation on chlorophyll content, which can further elucidate the mechanisms underlying these mutations [28]. Furthermore, the dosage and duration of radiation exposure may play a critical role in shaping the extent of chlorophyll mutations, indicating the complexity of this genetic response [29]. Understanding these genetic responses to gamma radiation is essential in both radiation biology and agriculture, where it can inform strategies for crop improvement and radiation safety.

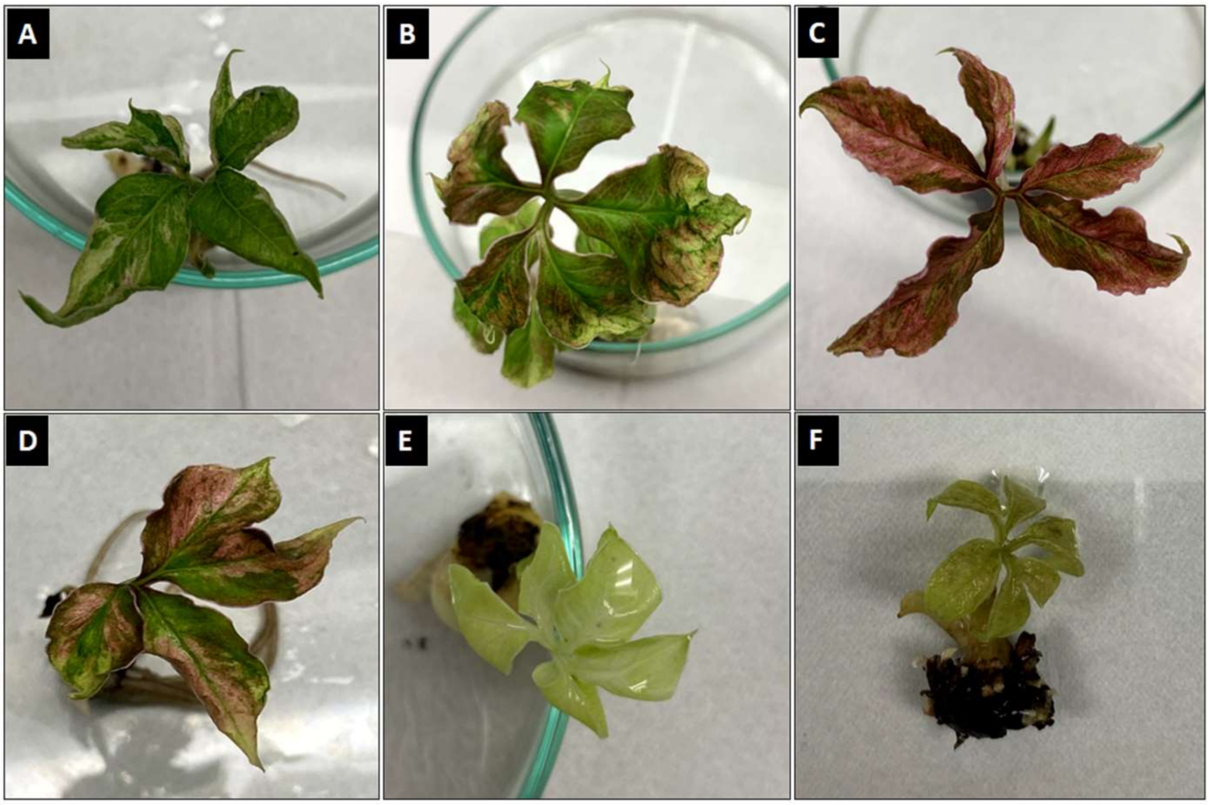

Figure 3. leaf chlorophyl mutant after gamma radiation. (a) white streak at 2 Gy, (b) red streak with wrinkled leaf edges at a dose of 4 Gy, (c,d) red streak at a dose 4 Gy, (e) albino at a dose of 2 Gy, (f) dwarf porang at dose 6 Gy.

In conclusion, gamma radiation success in generating new variation of porang indicating by the emerging of new morphological character of porang. However, the scientific discussion highlights the dual role of gamma radiation as a tool in plant science—offering the potential for enhancing desired morphological traits while carrying the risk of inducing undesirable effects. The careful selection of radiation doses emerges as a crucial consideration for researchers and agricultural practitioners seeking to harness this technology for the benefit of Porang cultivation and other plant-based applications. Moreover, these findings open avenues for future investigations aimed at unravelling the molecular and genetic mechanisms governing plant responses to radiation, ultimately advancing our ability to manipulate plant traits for agricultural and scientific purposes.

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