



# Elucidation of the Radiosensitivity Level of *Amorphophallus paeoniifolius* (Dennst.) Nicolson Embryogenic Callus Induced by Gamma Ray Irradiation <sup>†</sup>

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**Abstract:** The tuber of *Amorphophallus paeoniifolius* (Dennst.) Nicolson (Araceae) has been receiving attention as an alternative food source. However, the tuber contains oxalate compounds which reduce the utilization of this species as a food material. Inducing genetic mutations using gamma ray irradiation followed by a selection process can be used to increase genetic diversity and produce genetically improved cultivars of *A. paeoniifolius*. To achieve an effective mutation through gamma ray irradiation, the threshold of the sensitivity level of particular tissues is necessary to be elucidated in advance. Hence, the objective of the current study was to determine the level of radiosensitivity of in vitro-cultured *A. paeoniifolius* embryogenic callus to gamma rays. The main treatment factor in this experiment was different levels of gamma ray irradiation, including 0, 5, 10, 15, and 20 Gy. Plant growth parameters, such as the number of roots, shoots, and leaves, and also the height of plantlets arising from the callus, were decreased by applying gamma ray irradiation. Importantly, applying irradiation doses greater than 15 Gy significantly decreased the proportion of surviving embryogenic calluses. The lethal doses 20, 30, and 50 (LD<sub>20</sub>, LD<sub>30</sub>, and LD<sub>50</sub>) of calluses were 1.75, 5.44, and 12.84 Gy gamma irradiation, respectively. Since the high frequency of mutation was previously often found in around LD<sub>20</sub>–LD<sub>50</sub> irradiated callus in other plants, this present study suggested that the effective gamma irradiation of *A. paeoniifolius* embryogenic callus was between 1.75 Gy until 12.84 Gy.

**Keywords:** *Amorphophallus*; elephant foot yam; functional food; gamma ray irradiation; genetic diversity; lethal dose; mutation



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## 1. Introduction

*Amorphophallus paeoniifolius* (Dennst.) Nicolson belongs to Araceae and is widely distributed in Asia, Australia, and Africa. Within the Asian countries, the gene pool centers of this species are documented in Indonesia, Thailand, and India [1].

It has been previously reported that the tuber and other plant organs of *A. paeoniifolius* can be used as a source of medicine [2–5] or a raw food material [6–12]. Besides having a high potential utilization as both a medicine and food, the productivity of this crop is relatively high at approximately 50–80 ton ha<sup>−1</sup> [13]. However, the presence of acidity and oxalate compounds contributes to the underutilization of this plant as an alternative food source [14,15].

Various approaches have been previously recognized to improve the usability of the plant, such as altering the structures and properties of the *A. paeoniifolius* tuber by gamma ray irradiation, directly using the starch as a downstream material [6]. In the more upstream step, Abraham et al. [16] recently reported their efforts to improve the *A. paeoniifolius* characters by genetic transformation. Induced mutations by ionizing radiation such as gamma rays have been also widely used as one of the plant breeding methods to increase

genetic diversity and improve crop quantity and quality in numerous plants [17]. For example, the gamma ray irradiation technique was used for enhancing the genetic diversity and improving several crops, such as potato (*Solanum tuberosum* L.) [18], cassava (*Manihot esculenta*) [19], white taro (*Xanthosoma sagittifolium* (L.) Schott) [20], Bogor taro (*Colocasia esculenta* (L.) Schott) [21], rodent tuber (*Typhonium flagelliforme* (Lodd.) Blume) [22], sweet potato (*Ipomoea batatas* (L.) Lam.) [23], and *Amorphophallus muelleri* Blume [24]. To achieve an effective mutation through gamma ray irradiation, the threshold of the sensitivity level of particular tissues is necessary to be elucidated in advance. Hence, the objective of the current study was to determine the level of radiosensitivity of in vitro-cultured *A. paeoniifolius* embryogenic callus to gamma ray irradiation.

## 2. Experiments

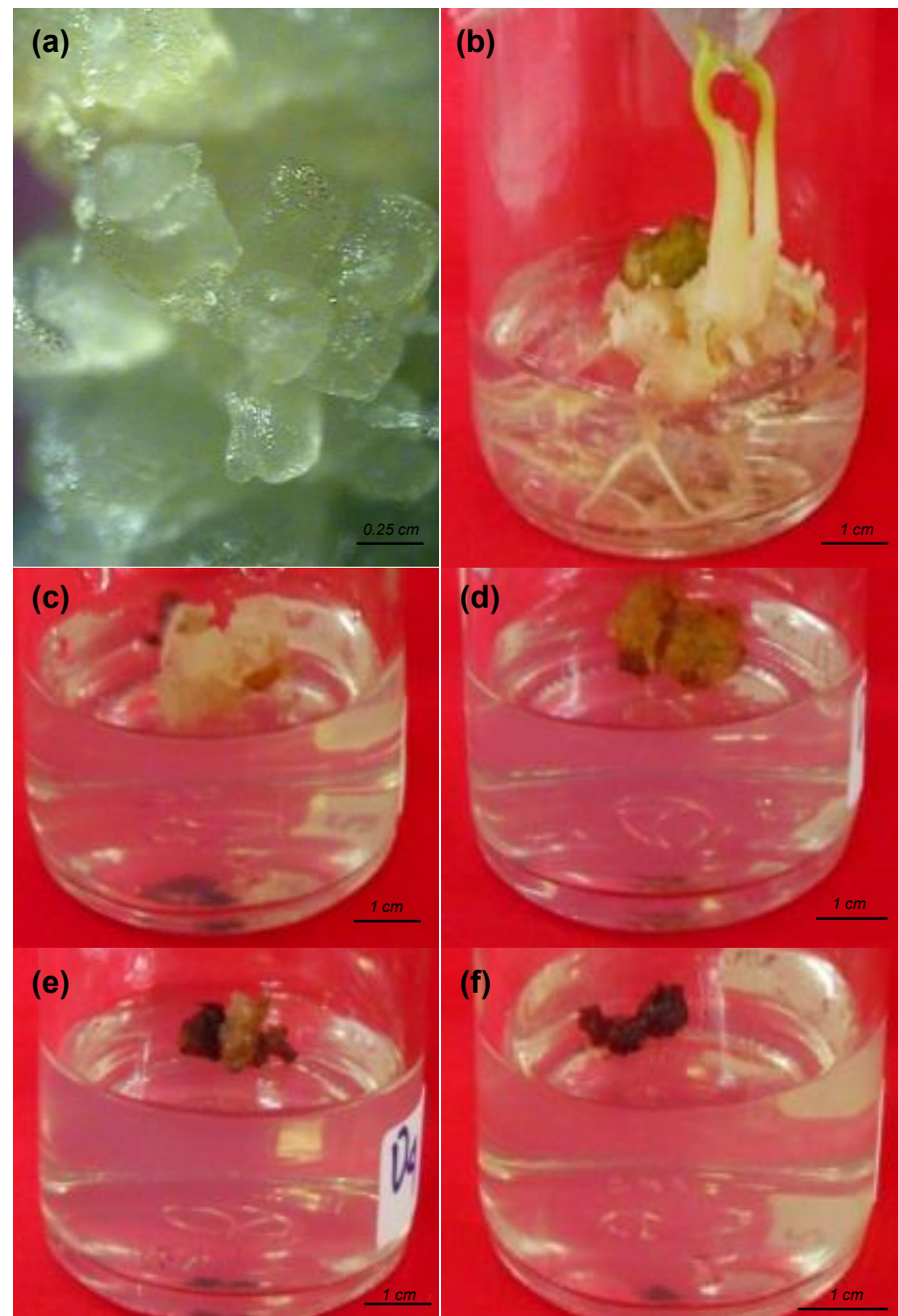
The explants were derived from the vigorous leaflets and petioles of *A. paeoniifolius* in vitro plantlets collection of the Plant Tissue Culture Laboratory, Research Center for Plant Conservation and Botanic Gardens, National Research and Innovation Agency of the Republic of Indonesia. *Amorphophallus paeoniifolius* embryogenic calluses were induced by using the modified full-strength in vitro culture Murashige and Skoog (MS) medium [25]. Tissue cultures were incubated at 23–25 °C room temperature with a 16/8 h light/dark cycle of 800–1000 lux in light.

The embryogenic calluses of *A. paeoniifolius* were exposed to a  $^{60}\text{Co}$  gamma ray irradiation (Gamma Chamber 4000A) at the Research and Technology Center for Application of Isotope and Radiation, National Research and Innovation Agency of the Republic of Indonesia. The calluses were treated with five irradiation levels, viz. 0, 5, 10, 15, and 20 Gy. For each treatment, fifty glass bottles containing a single embryogenic callus were irradiated. A completely randomized design was used for this experiment. The irradiated calluses were immediately transferred to the newly prepared modified MS medium. Afterwards, the subcultures were performed every month to keep up the nutrients available for the calluses. One month after irradiation, the calluses were both qualitatively and quantitatively observed. The survival rate of the irradiated callus was initially scored on a six-level callus color freshness scale (6 = green, 5 = light green, 4 = greenish white, 3 = brownish white, 2 = light brown, and 1 = brown). The survival percentage of callus and callus covered area was also analyzed. Importantly, the lethal dose level at 20, 30, and 50, or LD<sub>20</sub>, LD<sub>30</sub>, and LD<sub>50</sub>, respectively, of irradiated embryogenic calluses were quantitatively predicted by a linear curve estimation. Furthermore, the observation of numerous growth parameters of the plantlets arising from the embryogenic calluses were conducted two months after gamma ray irradiation treatment. Data were analyzed using analysis of variance by f-test at 5% significance level. Significantly different results were further analyzed by Duncan's Multiple Range Test (DMRT) with a 5% level of significance. Microsoft Excel 2013 and Statistical Tool for Agricultural Research (STAR) for analyzing the data were used.

## 3. Results and Discussion

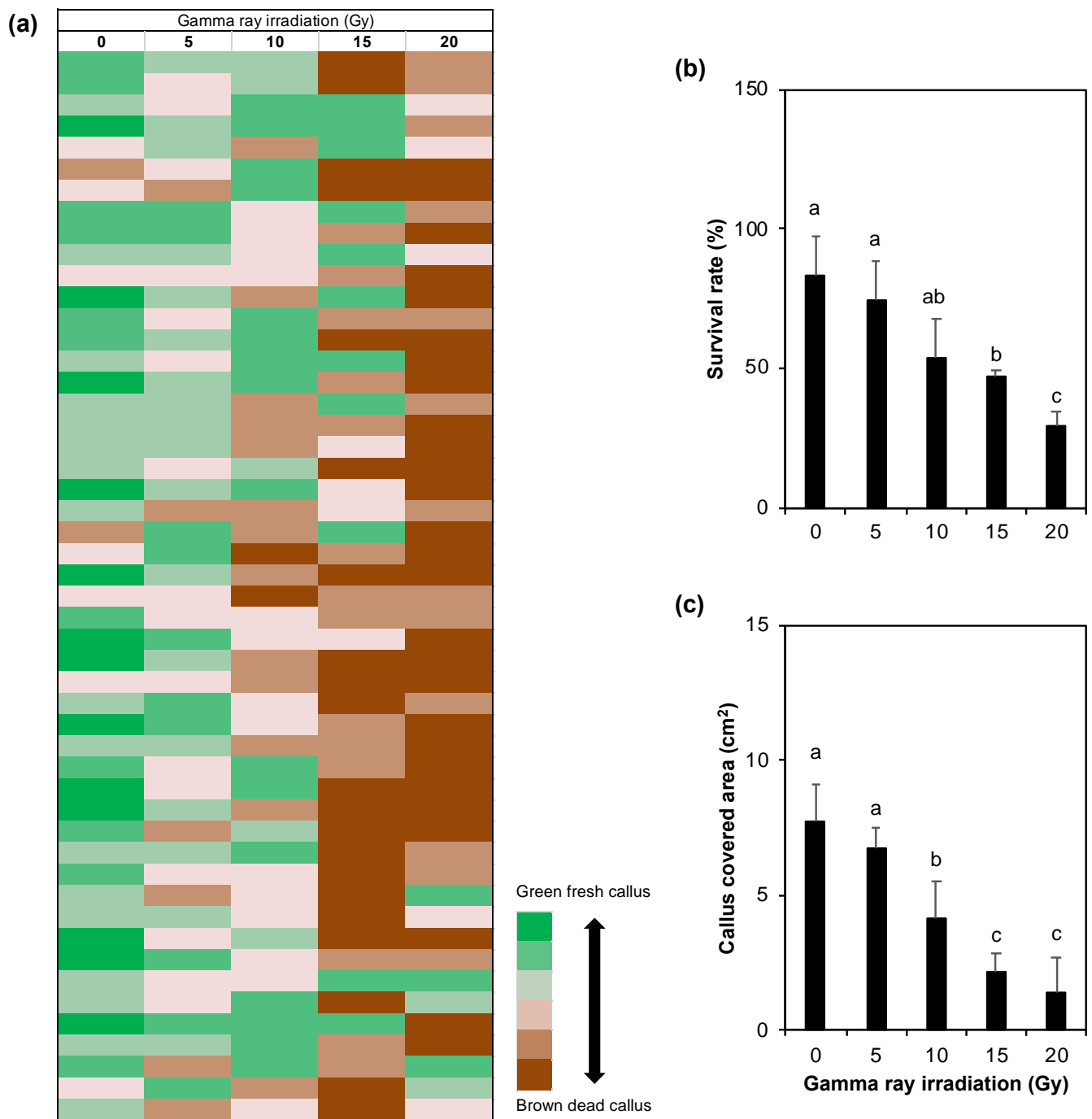
### 3.1. Embryogenic Callus Survival Rate

Embryogenic calluses of *A. paeoniifolius* were induced by tissue culture technique. The fresh and healthy embryogenic calluses were produced after six-months incubation (Figure 1a). The tissue culture method, as previously reported, could increase the plant genetic diversity through somaclonal variation [26–28]. Moreover, the combination of in vitro culture and exogenously applied mutations, such as gamma ray irradiation, could enhance the genetic diversity [17]. Hence, the calluses were exposed to five levels of gamma ray irradiation, namely 0, 5, 10, 15, and 20 Gy, as previously reported in another *Amorphophallus* species [24]. As shown in Figure 1b–f, the tissues of calluses were gradually damaged by increasing the level of gamma ray irradiation. The mechanism of tissue injury altered by external gamma ray irradiation has been comprehensively explained by Sparrow et al. [29].



**Figure 1.** Embryogenic callus of *Amorphophallus paeoniifolius*. (a) Before gamma ray irradiation at six months after cultivation, and at (b) 0 Gy, (c) 5 Gy, (d) 10 Gy, (e) 15 Gy, and (f) 20 Gy after one month gamma ray irradiation (seven months after cultivation).

To quantitatively measure the tissue destruction affected by gamma ray irradiation, the survival rate of the irradiated callus was objectively observed by six-level freshness scale scoring (6 = green fresh callus to 1 = brown dead callus). The heatmap illustration of each single callus condition among the treatments ( $n = 50$ ) is shown in Figure 2a. The proportion of survived callus was significantly decreased by applying 15 Gy gamma ray irradiation. In addition, application of 10 Gy substantially declined the callus covered area (Figure 2c). These results suggested that the 10–15 Gy was the critical irradiation level for *A. paeoniifolius* embryogenic callus survival rate.

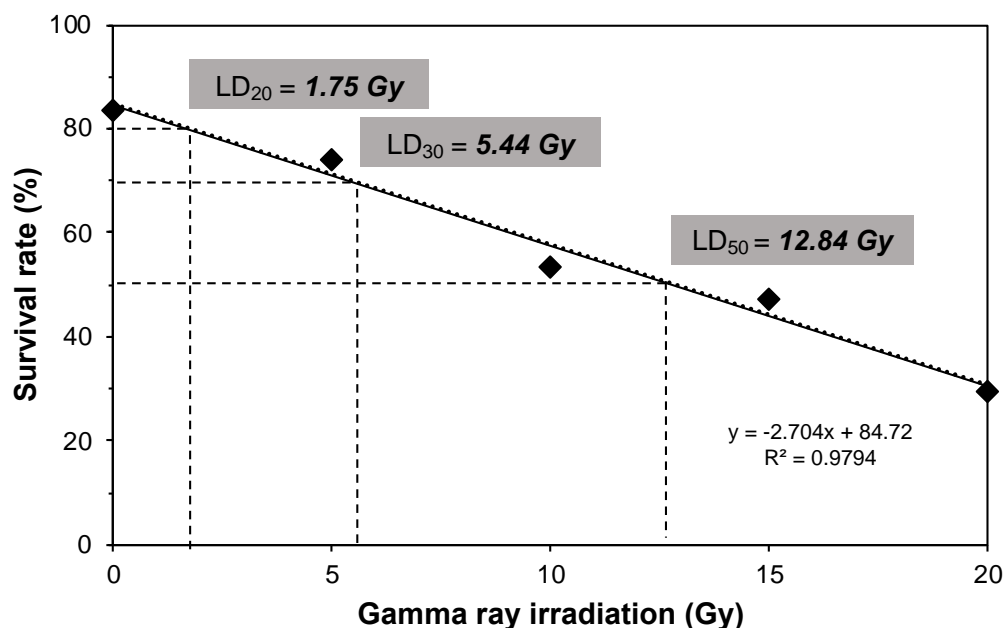


**Figure 2.** Survival rate of *Amorphophallus paeoniifolius* embryogenic callus. (a) Heatmap illustration of the callus condition, (b) survival percentage of the callus, and (c) callus covered area after one-month gamma ray irradiation (seven months after cultivation). Values are means  $\pm$  SD. Different letters mean significant difference. Duncan's Multiple Range Test,  $p < 0.05$  ( $n = 50$ ).

### 3.2. Radiosensitivity Level of the Embryogenic Callus

To further investigate the gamma ray irradiation-induced tissue destruction of *A. paeoniifolius* embryogenic calluses and to determine the LD<sub>20</sub>, LD<sub>30</sub>, and LD<sub>50</sub> (exposures required to decrease survival by 20, 30, and 50%), the radiosensitivity level of the calluses were analyzed using linear curve estimation as previously reported [30]. As shown in Figure 3, an estimation of LD<sub>20</sub>, LD<sub>30</sub>, and LD<sub>50</sub> of *A. paeoniifolius* embryogenic calluses from this study were 1.75 Gy, 5.44 Gy, and 12.84 Gy, respectively. In comparison with the

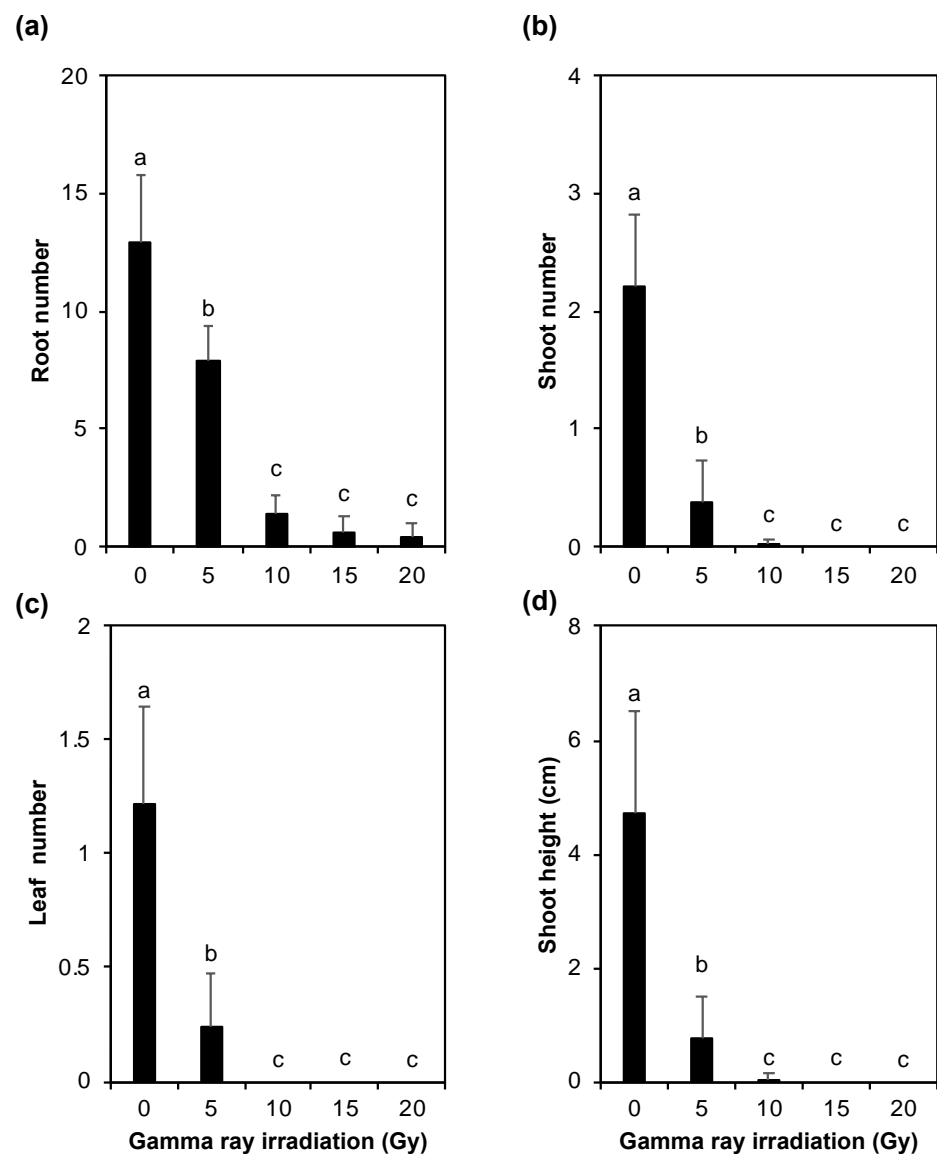
previous studies, the estimated LD<sub>20</sub>, LD<sub>30</sub>, and LD<sub>50</sub> of *Celosia argentea* L. in vitro plantlets were 35.65 Gy, 46.68 Gy, and 68.73 Gy, respectively [30]. Isnaini and Novitasari [31] predicted the LD<sub>50</sub> of two variants of *Nepenthes ampullaria* Jack in vitro plantlets were around 31.0 Gy and 41.6 Gy. These findings suggested that the lethal dose level affected by gamma ray irradiation differs among plant species and highly depend on tissues examined and developmental stages. Since the high frequency of mutation was previously often found in around LD<sub>20</sub>–LD<sub>50</sub> irradiated tissues in other herbaceous and crop species [30,32], this present study suggested that the effective gamma irradiation of *A. paeoniifolius* embryogenic callus was between 1.75–12.84 Gy.



**Figure 3.** Estimated lethal dose (LD) values of *Amorphophallus paeoniifolius* calluses after one-month gamma ray irradiation (seven months after cultivation).

### 3.3. Growth of Plantlets

In general, the gamma ray irradiation inhibited the growth and development of plantlets arising from the embryogenic calluses of *A. paeoniifolius* (Figure 4). The number of roots, shoots, and leaves, and the shoot height were significantly reduced by 5 Gy gamma ray irradiation (Figure 4). The growth inhibition caused by gamma ray irradiation was more remarkably found in 10–20 Gy treatments, suggesting that the *A. paeoniifolius* tissues from embryogenic calluses were highly sensitive to the gamma ray irradiation.



**Figure 4.** Plantlet growth arising from the embryogenic callus of *Amorphophallus paeoniifolius*. (a) Root number, (b) shoot number, (c) leaf number, and (d) shoot length after two-months gamma ray irradiation (eight months after cultivation). Values are means  $\pm$  SD. Different letters mean significant difference. Duncan's Multiple Range Test,  $p < 0.05$  ( $n = 50$ ).

#### 4. Conclusions

The exposures required from gamma ray irradiation to decrease the survival rate of *A. paeoniifolius* embryogenic calluses by 20, 30, and 50% were 1.75 Gy, 5.44 Gy, and 12.84 Gy, respectively. Thus, the findings indicated an effective range of irradiation level which possibly induce *A. paeoniifolius* embryogenic calluses mutation was around 1.75–12.84 Gy. In this study, the resulted potential mutant has not been recognized, and further study on that aspect is necessary in the near future.

**Author Contributions:** R.R.R. and Y. conceived the study. R.R.R. designed the experiments. R.R.R. and Y.I. performed the experiments. R.R.R. analyzed the data. R.R.R. wrote the manuscript with help from all the others. R.R.R. is a main contributor for this work. All authors have read and agreed to the published version of the manuscript.

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