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Proceeding Paper

Elucidation of the Radio-Sensitivity Level of *Amorphophallus paeoniifolius* (Dennst.) Nicolson Embryogenic Callus Induced by Gamma Ray Irradiation †

Reza Ramdan Rivai *, Yupi Isnaini and Yuzammi

Research Center for Plant Conservation and Botanic Gardens, National Research and Innovation Agency of the Republic of Indonesia, Bogor, West Java, Indonesia; yupinurfauzi@gmail.com (Y.I.); yuzammi@yahoo.co.id (Y.)

* Correspondence: rezaramdanrivai@gmail.com or reza.ramdan.rivai@lipi.go.id

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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 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 tissue is necessary to be elucidated in advance. Hence, the objective of the current study was to determine the level of radio-sensitivity 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, shoot, and leaves, also height of plantlets arising from the callus were declined by applying gamma ray irradiation. Importantly, applying irradiation doses greater than 15 Gy significantly decreased the proportion of survived embryogenic callus. The lethal doses 20, 30, and 50 (LD_{20}, LD_{30}, and, LD_{50}) of calluse 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_{20}–LD_{50} 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 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 food raw material [6–12]. Besides a high potential utilization as both medicine and food, the productivity of this crop is relatively high, approximately 50–80 ton ha^{-1} [13]. However, the presence of acridity 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 *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 plant breeding method 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 (*Xhanthosoma 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 tissue is necessary to be elucidated in advance. Hence, the objective of the current study was to determine the level of radio-sensitivity 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}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 by 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 monthly performed to keep the nutrients availability for the calluses. One month after irradiation, the calluses were both qualitatively and quantitively observed. The survival rate of the irradiated callus was initially scored on a six-level of 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 were also analyzed. Importantly, the lethal dose level at 20, 30, and, 50 or LD_{20}, LD_{30}, and LD_{50}, respectively, of irradiated embryogenic calluses were quantitively predicted by a linear curve estimation. Furthermore, the observation of numerous growth parameters of the plantlets arising from the embryogenic calluses were conducted at 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 was previously reported that could increase the plant genetic diversity through somaclonal variation [26–28]. Moreover, the combination between 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 level of gamma ray irradiation namely 0, 5, 10, 15, 20 as previously reported in another *Amorphophallus* species [24]. As shown in Figure 1b–f, the tissues of calluses were

apparently and gradually damaged by increasing the level of gamma ray irradiation. The mechanism of tissues injury altered by external gamma ray irradiation has been comprehensively explained by Sparrow et al. [29].

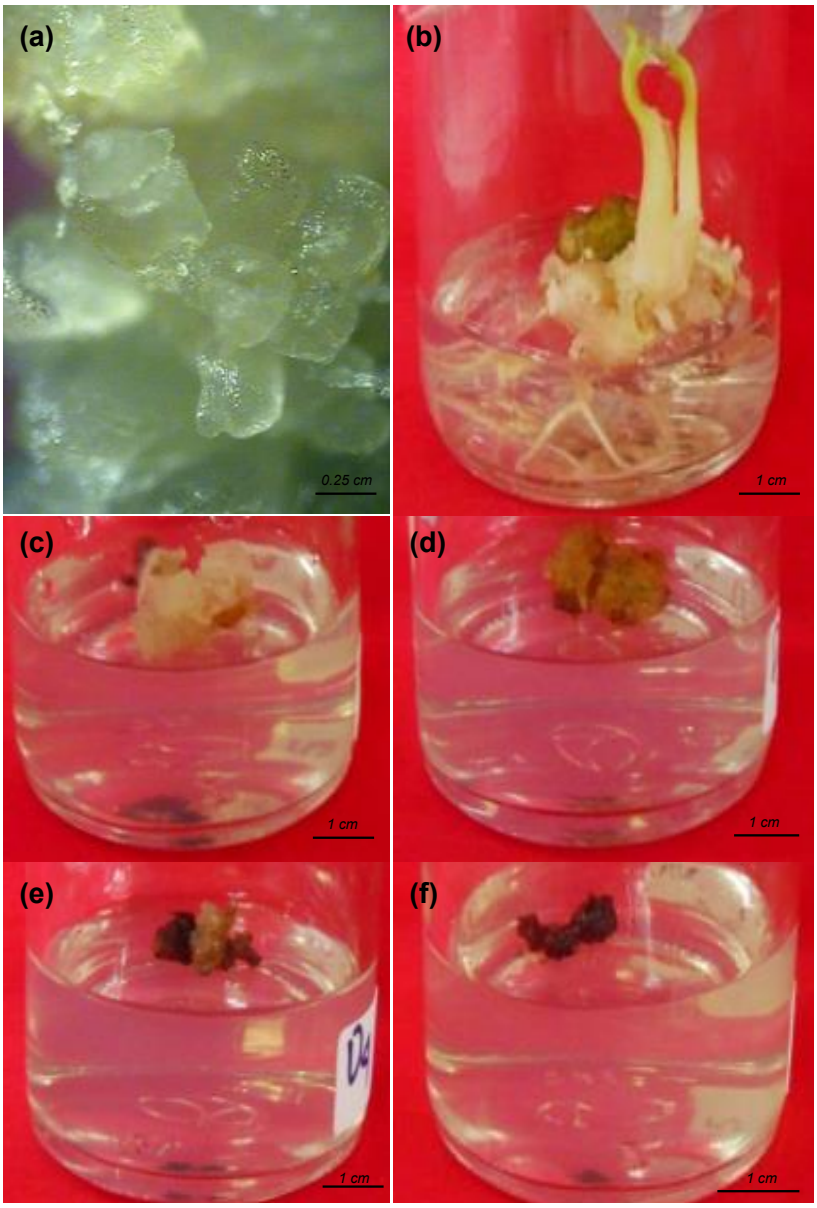

Figure 1. Embryogenic callus of *Amorphophallus paeoniifolius*. (**a**) Before gamma ray irradiation at six months after cultivation, and at (**b**) 0 Gy, (**c**) 5 Gy, (**d**) 10 Gy, (**e**) 15 Gy, and (**f**) 20 Gy after one month gamma ray irradiation (seven months after cultivation).

To quantitatively measure the tissues 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.

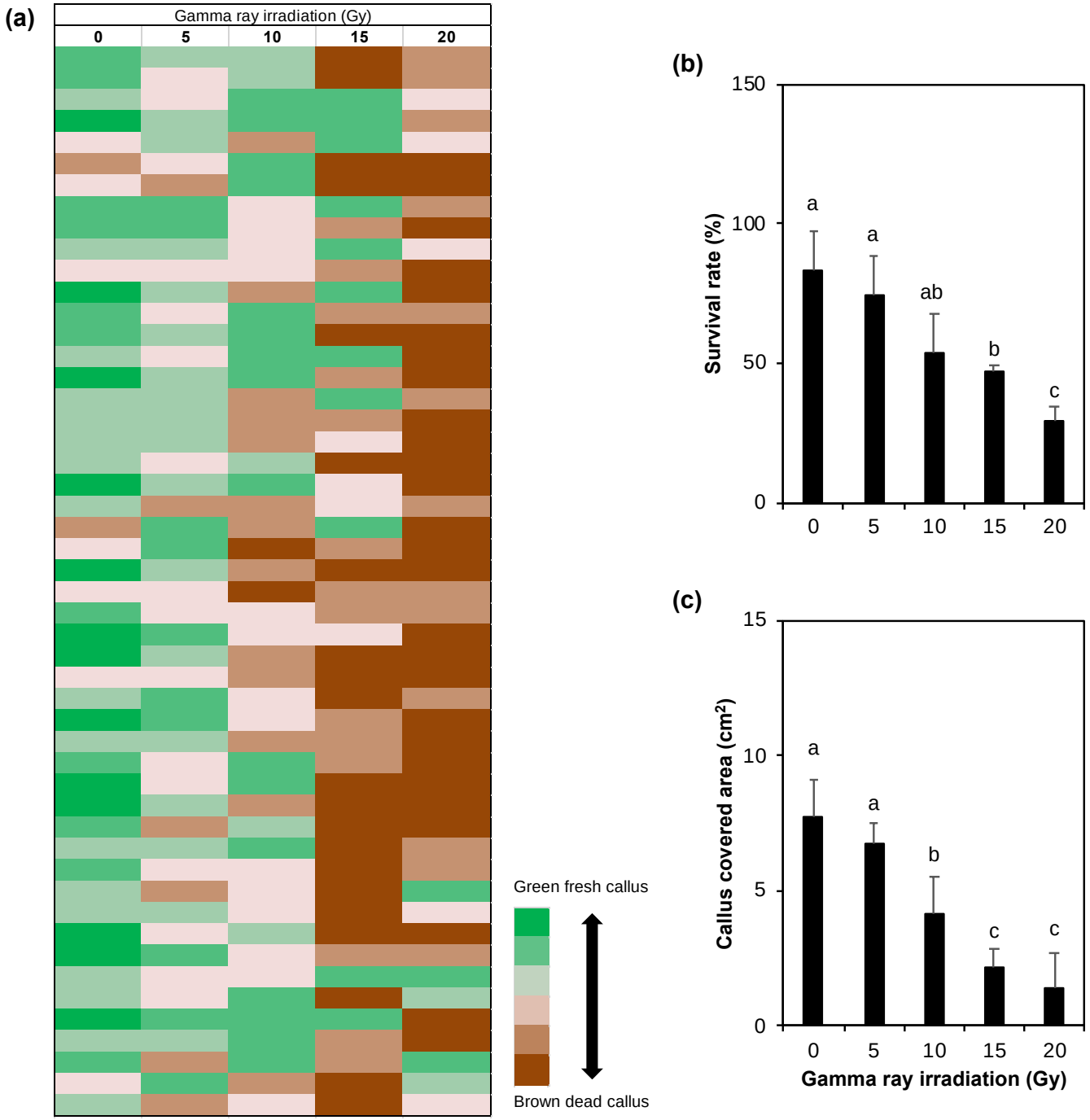

Figure 2. Survival rate of *Amorphophallus paeoniifolius* embryogenic callus. (**a**) Heatmap illustration of the callus condition, (**b**) survival percentage of the callus, and (**c**) callus covered area after one-month gamma ray irradiation (seven months after cultivation). Values are means ± SD.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

3.2. Radio-Sensitivity Level of the Embryogenic Callus

To further investigate the gamma ray irradiation-induced tissues destruction of *A. paeoniifolius* embryogenic calluses and to determine the LD_{20}, LD_{30}, and LD_{50} (exposures required to decrease survival by 20, 30, and 50 %), therefore the radio-sensitivity level of the calluses were analyzed using linear curve estimation as previously reported [30]. As shown in Figure 3, an estimation of LD_{20}, LD_{30}, and LD_{50} 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_{20}, LD_{30}, and LD_{50} of *Celosia argentea* L. in vitro

plantlets were 35.65 Gy, 46.68 Gy, and 68.73 Gy, respectively [30]. Isnaini & Novitasari [31] predicted the LD_{50} of two variants *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_{20}-LD_{50} 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.

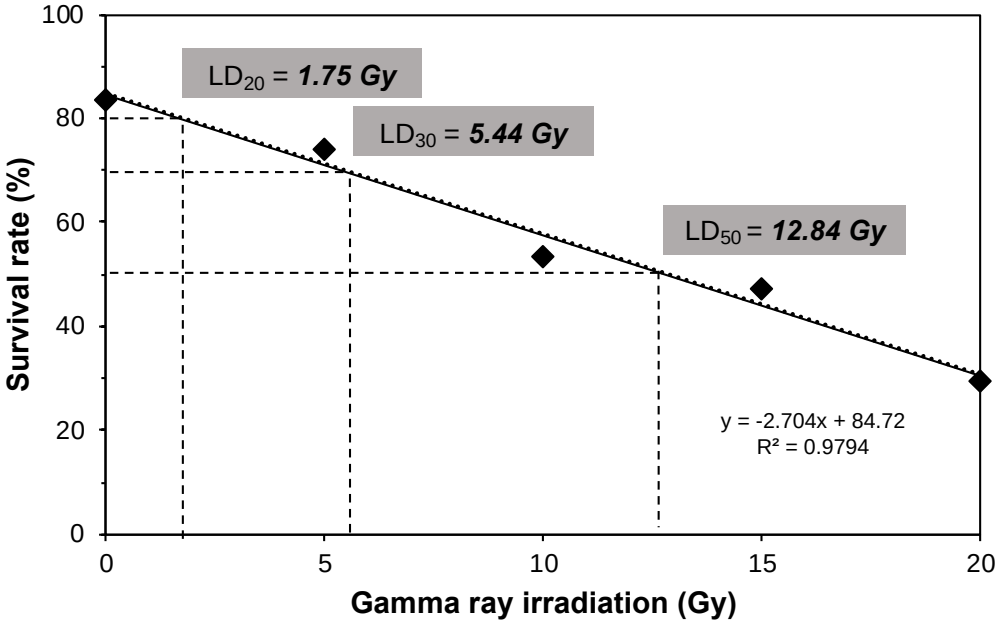

Figure 3. Estimated lethal dose (LD) values of *Amorphophallus paeoniifolius* calluses after one month gamma ray irradiation (seven months after cultivation).

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, shoot, leaves, and shoot height were significantly reduced by 5 Gy gamma ray irradiation (Figure 4). The growth inhibition caused by gamma ray irradiation was more remarkedly found in 10–20 Gy treatments. Suggesting that the *A. paeoniifolius* tissues from embryogenic calluses were highly sensitive to the gamma ray irradiation.

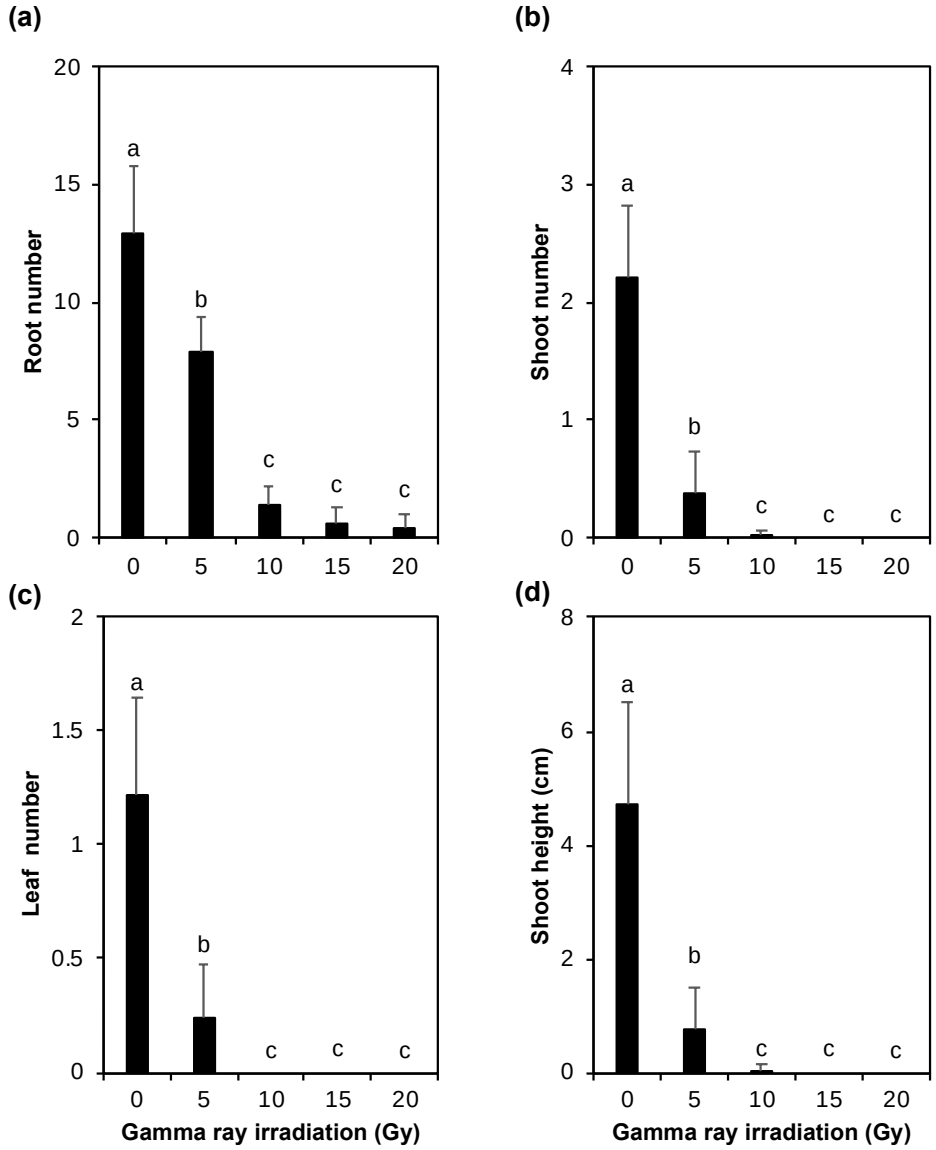

Figure 4. Plantlets growth arising from the embryogenic callus of *Amorphophallus paeoniifolius*. (**a**) Root number, (**b**) shoot number, (**c**) leaf number, and (**d**) shoot length after two months gamma ray irradiation (eight months after cultivation). Values are means ± SD. Different letters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

4. Conclusions

The exposures required from gamma ray irradiation to decrease 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 Gy–12.84 Gy. In this study, the resulted potential mutant has not been recognized, 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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References

1. Santosa, E.; Lian, C.L.; Sugiyama, N.; Misra, R.S.; Boonkorkaew, P.; Thanomchit, K. Population structure of elephant foot yams (*Amorphophallus paeoniifolius* (Dennst.) Nicolson) in Asia. *PLoS ONE* **2017**, 12, e0180000, doi:10.1371/journal.pone.0180000.
2. Angayarkanni, J.; Ramkumar, K.M.; Priyadharshini, U.; Ravendran, P. Antioxidant potential of *Amorphophallus paeoniifolius* in relation to their phenolic content. *Pharm. Biol*. **2010**, *48*, 659–665, doi:10.3109/13880200903241846.
3. Hurkadale, P.J.; Shelar, P.A.; Palled, S.G.; Mandavkar, Y.D.; Khedkar, A.S. Hepatoprotective activity of *Amorphophallus paeoniifolius* tubers against paracetamol-induced liver damage in rats. *Asian. Pac. J. Trop. Biomed.* **2012**, *2*, S238–42.
4. Morvin, Y.J.E.; Prabhu, S.; Vijayakumar, S. An ethnobotanical study of medicinal plants used by traditional healers in silent valley of Kerala, India. *J. Ethnopharmacol.* **2014**, *154*, 774–789, doi:10.1016/j.jep.2014.05.004.
5. Dey, Y.N.; Wanjari, M.M.; Kumar, D.; Lomash, V.; Jadhav, A.D. Curative effect of *Amorphophallus paeoniifolius* tuber on experimental hemorrhoids in rats. *J. Ethnopharmacol.* **2016**, *192*, 183–191, doi:10.1016/j.jep.2016.07.042.
6. Reddy, C.K.; Suriya, M.; Vidya, P.V.; Vijina, K.; Haripriya, S. Effect of γ-irradiation on structure and physico-chemical properties of *Amorphophallus paeoniifolius* starch. *Int. J. Biol. Macromol.* **2015**, *79*, 309–315, doi:10.1016/j.ijbiomac.2015.05.003.
7. Sukhija, S.; Singh, S.; Riar, C.S. Effect of oxidation, cross-linking and dual modification on physicochemical, crystallinity, morphological, pasting and thermal characteristics of elephant foot yam (*Amorphophallus paeoniifolius*) starch. *Food Hydrocoll.* **2016**, *55*, 56–64, doi:10.1016/j.foodhyd.2015.11.003.
8. Suriya, M.; Rajput, R.; Reddy, C.K.; Haripriya, S.; Bashir, M. Functional and physicochemical characteristics of cookies prepared from *Amorphophallus paeoniifolius* flour. *J. Food Sci. Technol.* **2017**, *54*, 2156–2165.
9. Suriya, M.; Reddy, C.K.; Haripriya, S.; Harsha, N. Influence of debranching and retrogradation time on behavior changes of *Amorphophallus paeoniifolius* nanostarch. *Int. J. Biol. Macromol.* **2018**, *120*, 230–236, doi:10.1016/j.ijbiomac.2018.08.059.
10. Yuzammi. The diversity of aroids (Araceae) in Bogor Botanic Gardens, Indonesia: Collection, conservation and utilization. *Biodiversitas* **2018**, *19*, 140–152, doi:10.13057/biodiv/d190121.
11. Nagar, M.; Sharanagat, V.S.; Kumar, Y.; Singh, L.; Mani, S. Influence of xanthan and agar-agar on thermo-functional, morphological, pasting and rheological properties of elephant foot yam (*Amorphophallus paeoniifolius*) starch. *Int. J. Biol. Macromol*. **2019**, *136*, 831–838, doi:10.1016/j.ijbiomac.2019.06.133.
12. Mutaqin, A.Z.; Kurniadie, D.; Iskandar, J.; Nurzaman, M.; Partasasmita, R. Ethnobotany of suweg, *Amorphophallus paeoniifolius*: Utilization and cultivation in West Java, Indonesia. *Biodiversitas* **2020**, *21*, 1635–1644.
13. Ravi, V.; Ravindran, C.; Suja, G. Growth and productivity of elephant foot yam (*Amorphophallus paeoniifolius* (Dennst.) Nicolson): An overview. *J. Root Crop* **2009**, *35*, 131–142.
14. Bradbury, J.H.; Nixon, R.W. The acridity of raphides from the edible aroids. *J. Sci. Food Agric*. **1998**, *76*, 608–616.
15. Singh, A.K.; Chaurasiya, A.K.; Mitra, S. Oxalate content in elephant foot yam (*Amorphophallus paeoniifolius* Dennst.-Nicolson) dry and fry cubes. *J. Pharma. Phytochem*. **2018**, *7*, 2905–2909.
16. Abraham, L.N.; Kamala, S.; Sreekumar, J.; Makeshkumar, T. Optimization of parameters to improve transformation efficiency of elephant foot yam (*Amorphophallus paeoniifolius* (Dennst.) Nicolson. *3 Biotech* **2021**, *11*, 1–8, doi:10.1007/s13205-021-02824-6.
17. Ahloowalia, B.S.; Maluszynski, M.; Nichterlein, K. Global impact of mutation-derived varieties. *Euphytica* **2004**, *135*, 187–204.
18. Cheng, L.; Yang, H.; Lin, B.; Wang, Y.; Li, W.; Wang, D.; Zhang, F. Effect of gamma-ray radiation on physiological, morphological characters and chromosome aberrations of minitubers in *Solanum tuberosum* L. *Int. J. Radiat. Biol.* **2010**, *86*, 791–799.
19. Khumaida, N.; Maharani, S.; Ardie, S.W. The leaf color performance on several lines of cassava and its relation with tuber yield as early reference. *Procedia Environ. Sci.* **2015**, *24*, 39–46, doi:10.1016/j.proenv.2015.03.007.
20. Fadli, N.; Syarif, Z.; Satria, B.; Akhir, N. The effect of gamma Cobalt-60 ray irradiation on cultivar growth in taro white (*Xhanthosoma Sagittifolium* L.). *Int. J. Environ. Agric. Biotechnol*. **2018**, *3*, 2020–2025.
21. Nurilmala, F.; Hutagaol, R.P.; Widhyastini, I.M.; Widyastuti, U.; Suharsono, S. Somaclonal variation induction of bogor taro (*Colocasia esculenta*) by gamma irradiation. *Biodiversitas* **2017**, *18*, 28–33.
22. Sianipar, N.F.; Maarisit, W. Detection of gamma-irradiated mutant of rodent tuber (*Typhonium flagelliforme* Lodd.) in vitro culture by RAPD molecular marker. *Procedia Chem*. **2015**, *14*, 285–294, doi:10.1016/j.proche.2015.03.040.

23. Kuranouchi, T.; Kumazaki, T.; Kumagai, T.; Nakatani, M. Breeding erect plant type sweetpotato lines using cross breeding and gamma-ray irradiation. *Breed Sci*. **2016**, *66*, 456–461.
24. Poerba, Y.S.; Imelda, M.; Wulansari, A.; Martanti, D. Induksi mutasi kultur in vitro *Amorphophallus muelleri* Blume dengan irradiasi gamma (in Indonesian). *J. Tek. Ling*. **2009**, *10*, 355–364.
25. Murashige, T.; Skoog, F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiol Plant* **1962**, *15*, 473–497, doi:10.1111/j.1399-3054.1962.tb08052.x.
26. Karp, A. Mechanisms of somaclonal variation. *Biotechnol. Biotechnol. Equip.* **1993**, *7*, 20–25.
27. Hutami, S.; Mariska, I.; Supriati, Y. Peningkatan keragaman genetik tanaman melalui keragaman somaklonal (in Indonesian). *J. AgroBiogen* **2016**, *2*, 81.
28. Krishna, H.; Alizadeh, M.; Singh, D.; Singh, U.; Chauhan, N.; Eftekhari, M.; Sadh, R.K. Somaclonal variations and their applications in horticultural crops improvement. *3 Biotech* **2016**, *6*, 54.
29. Sparrow, A.H.; Schwemmer, S.S.; Bottino, P.J. Effects of external gamma radiation from radioactive fallout on plants with special reference to crop production. *Radiat. Bot.* **1971**, *11*, 85–118.
30. Hayati, D.; Aisyah, S.I.; Krisantini. Radiosensitivity levels of in vitro cultured *Celosia cristata* planlets by γ—ray irradiation. *J. Trop. Crop Sci.* **2016**, *3*, 61–65.
31. Isnaini, Y.; Novitasari, Y. Penentuan kisaran dosis iradiasi gamma optimal dalam pemuliaan mutasi *Nepenthes ampullaria* Jack. secara in vitro (in Indonesian). *J. Ilm. Apl. Isot. dan Radiasi* **2020**, *16*, 15–22.
32. Astuti, D.; Sulistyowati, Y.; Nugroho, S. Uji radiosensitivitas sinar gamma untuk menginduksi keragaman genetik sorgum berkadar lignin tinggi (in Indonesian). *J. Ilm. Apl. Isot. dan Radiasi* **2019**, *15*, 1–6.