Jurnal Kultivasi Vol. 23 (2) August 2024
ISSN: 1412-4718, eISSN: 2581-138x

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The effect of various doses of gamma ray irradiation on growth and chlorophyll changes of three adlay genotypes

Abstract. Adlay has great potential to be developed into an alternative food source in Indonesia because it contains carbohydrates and high fat. The aim was to effect of various doses of gamma-ray irradiation on growth, chlorophyll changes, and determination of lethal dose (LD_{50}) three adlay genotypes in M_1 generation. This study was conducted by using the experimental quantitative method without design. The material used consists of three adlay genotypes, including genotypes #28, #37, and #38. The seeds were treated with 0 Gy (control), 100 Gy, 200 Gy, 300 Gy, 400 Gy, and 500 Gy doses of gamma-ray irradiation by the Research Center for Radiation Process Technology, the National Research and Innovation Agency using Gamma Cell 220. The research was conducted at the Faculty of Agriculture Experimental Station, Universitas Padjadjaran, from December 2017 to July 2018. The LD_{50} was calculated by the curve-fitting analysis program based on characters of survival percentage, height seedling, root length, and appearance of leaf chlorophyll change in the three adlay genotypes. The results showed that the treatment of gamma-ray irradiation gave a diverse response to characters of survival percentage, height seedling and rooting length of the three adlay genotypes. There were LD_{50} for each genotype, 346 Gy for genotype #28, 381 Gy for genotype #37, and 371 Gy for genotype #38. The optimum dose of gamma-ray irradiation for the three adlay genotypes was 300 Gy. The appearance of chlorophyll change leaf the three adlay genotypes caused by gamma ray irradiation treatments with doses of 100 - 500 Gy were able to produce variation in the spectrum and frequency of different chlorophyll change in the M_1 generation, the irradiation treatment of 400 Gy dose was able to make the highest frequency of chlorophyll mutations with a total frequency of 46.28%. Meanwhile, the gamma-ray irradiation treatment of 300 Gy produced the broadest chlorophyll mutant spectrum with 6 types of chlorophyll change consisting of tigrina, striata, viridis, variegata, maculata and albina green.

Keywords: Adlay · Chlorophyll change · Dose gamma ray

Submitted: 5 August 2024, Accepted: 14 August 2024, Published: 14 August 2024
DOI: https://doi.org/10.24198/kultivasi.v23i2.55619

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Qosim WA, Anas, Rachmadi M, Amien S, Islami RZ, Ramdani S. 2024. The effect of various doses of gamma ray irradiation on growth and chlorophyll changes of three adlay genotypes. Jurnal Kultivasi, 23(2): 209-216.

ISSN: 1412-4718, eISSN: 2581-138x

Introduction

Adlay (*Coix lacryma-jobi* L.) is an underutilized crop which has a great potency to be developed as an alternative and functional food (Suyadi et al., 2019). Adlay is used both as a food and traditional medicine and contains high nutritional components and medicinal value (Feng et al., 2020). Adlay contains protein, fat, vitamin B_1, and higher calcium levels than rice, corn, and sorghum (Din et al., 2021). In addition, adlay is also a plant that is easy to cultivate, resistant to pests and diseases, drought tolerant, and widely adapted to various environmental conditions (Masahid et al., 2021). Adlay can be used as an alternative food in the form of rice (broken seeds). Adlay rice can be processed into adlay porridge, adlay flour which can be used to make brownies, various pastries, and snacks. This can accelerate the achievement of diversification of food and to support national food security.

Mutagenesis can be used as an effective tool to improve and modify genotypes of popular cultivars suitable for modern agricultural and commercial needs (Andrew-Peter-Leon et al., 2021). Gamma irradiation has been used as a physical mutagen to produce various food crop cultivars including rice (*Oryza sativa* L.) (Malhotra, 2023), wheat (*Triticum aestivum* L.) (Bayarsaikhan et al., 2022), sorghum (*Sorghum bicolor* L.) (Human et al., 2020), and adlay (Nakagawa & Kato, 2017). Several studies have shown that the use of gamma-ray irradiation at low doses can induce changes in physiology and biochemistry, resulting in faster vegetative growth and earlier flowering. Gamma-ray irradiation can increase production, early maturity, cold resistance, pathogens, fall, more dwarf, and better seed quality (Vrinten et al., 1999). The relation between various doses and chlorophyll changes is very important, because increasing doses can increase chlorophyll changes. Mutation induction in cereal crops such as rice, corn, sorghum, and wheat has been widely carried out, but not much has been done especially adlay in Indonesia. Information about the effective dose of gamma-ray irradiation to induce mutations adlay is not yet available so a dose-oriented test is needed first. The aim is to the effect of various doses of gamma-ray irradiation on growth, chlorophyll changes, and determination of LD_{50} three adlay genotypes in M_1 generation.

Materials and Methods

The material used was adlay type, consisting of three genotypes namely, #28, #37, and #38 (Collection of Plant Breeding Laboratory). The three adlay genotypes have potential to improve in West Java. Seeds of adlay variety were treated with gamma-ray irradiation from Cobalt-60 sources (Gamma Cell type 220) at Research Center for Radiation Process Technology, the National Research and Innovation Agency, Pasar Jumat, Jakarta, and were planted at Ciparanje Experimental Station, Faculty of Agriculture, Universitas Padjadjaran, Jatinangor, with the altitude of ± 750 meters in above sea level (m asl). This experiment was conducted from December 2017 to July 2018.

The experiment was quantitative methods without design. Because of effect, gamma ray irradiation caused different constitution genetic. Adlay seeds were irradiated with six dose levels consisting of 0 Gy (control), 100 Gy, 200 Gy, 300 Gy, 400 Gy, and 500 Gy. There was no seed treatment before and after treated gamma-ray irradiation, because the seeds had high germination. The radiosensitivity test was carried out by planting 300 seeds from each irradiation treatment and control in a germination box containing soil mixed with manure (1:1) with 3 replications in the Plastic House. Survival percentage of seedlings, height of seedling, and root length were measured 3 weeks after seed sowing. The optimal irradiation doses were determined based on Lethal Dose (LD). The value of LD_{50} was determined based on the regression equation using the mycurvefit.com program.

Planting the M_1 generation in the field was carried out at the end of the dry season. Three-week-old seedlings were transferred to the field in experimental plots with a spacing of 60 cm x 40 cm. Manure as basic fertilizer was given as much as 5 tons/ha. Fertilization uses NPK 16-16-16 fertilizer as much as 200 kg/ha. Plant maintenance including irrigation, weed control, and pest and disease control was carried out to obtain optimal plant growth results. Observation of chlorophyll change that occurs has been calculated based on the following formula (Prina & Pravet 1988):

$$\text{Frequency mutations} = \frac{\textbf{\textit{Plant chlorophyll mutation}}}{\textbf{\textit{Number of plant mutation}}}$$

Results and Discussion

Based on the results of the study showed that the survival percentage of three adlay genotypes decreased with increasing doses of gamma-ray irradiation (Figure 1). The decrease in the survival percentage is due to the deterministic effect. Mutation breeding induced by ionizing radiation has been proven to increase plant chromosomal variation, cause changes in morphology, physiology, and biochemistry, and breed new varieties in a short cycle (Su et al., 2019).

The deterministic effect occurs when the irradiation dose received by the plant is above the threshold dose. The higher the dose of gamma irradiation given, the more severe the damage effects caused and the lower the chance of plants to grow or germinate. In this experiment, LD_{50} obtained based on the survival percentage of each genotype was 346 Gy at #28 genotype, 381 Gy at genotype #37, and 371 Gy at genotype #38. Differences in LD_{50} values indicate that the radiosensitivity to gamma rays was genotype-dependent. Genotype #28 has the highest radiosensitivity compared to genotypes #37 and #38, because of the different background genetic three adlay genotypes.

The decrease in germination is likely caused by the effect of mutagens on the meristematic tissue of the seeds as well as chromosomal aberrations and interference with DNA replication and growth regulators (Asare et al., 2017). Furthermore, at certain doses, the seeds are able to germinate but cannot survive due to DNA damage and are unable to repair (Sood et al., 2016). High doses of gamma irradiation can damage the integrity and permeability of cell membranes, causing inhibition of nutrient and water absorption, thereby disrupting plant growth and physiological activity (Li et al., 2021). Several factors affect the radiosensitivity of plants to irradiation, namely the first physical factors such as morphological forms of plant material can affect cell physical resistance when receiving light irradiation gamma, the second is biological factors such as genetic factors, and the third are environmental factors such as oxygen, moisture content, post-irradiation storage, and temperature. The response of survival percentage, seedling height, and root length decreases linearly with increasing dose of gamma-ray irradiation compared to the control (Figure 2). The maximum seedling height and root length were recorded in the control (0 Gy), while the seedling height exposed to 100 – 500 Gy decreased by 21 – 93% at genotype #28, 18 – 92% at genotype #37, and 19 – 92% at genotype #38, and the root length with the same dose decreased by 5 – 85% at genotype #28, 11 – 86% at genotype #37, and 17 – 85% at genotype #38. This result is in line with the research of on local rice in West Sumatra (Hayati et al. 2014).

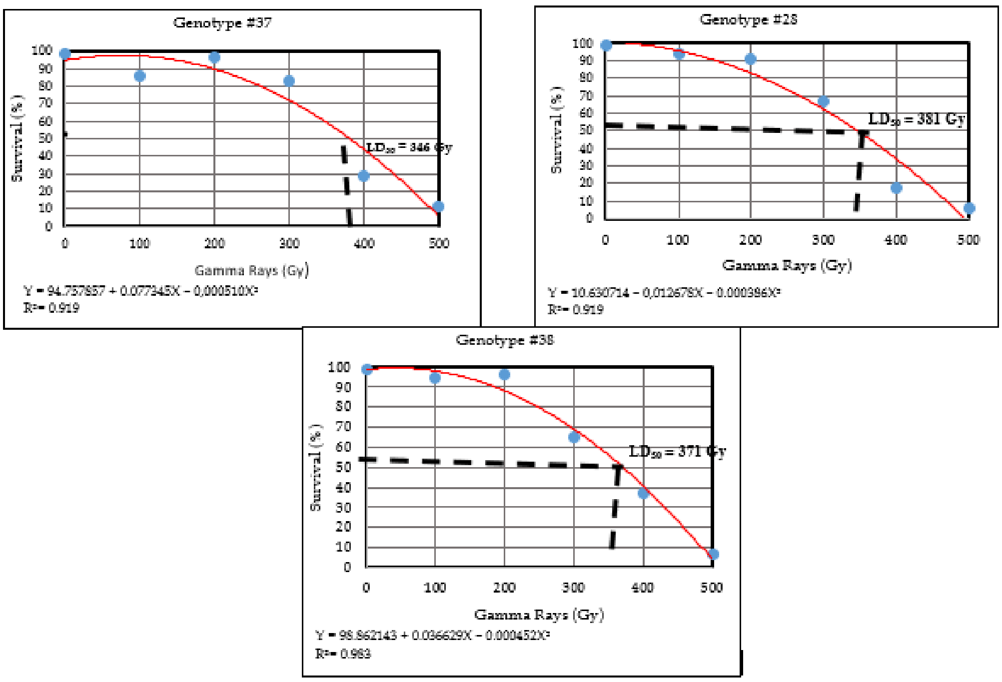

Figure 1. The effect of gamma irradiation on survival percentage of three adlay genotypes at 21 days after planting (DAP)

ISSN: 1412-4718, eISSN: 2581-138x

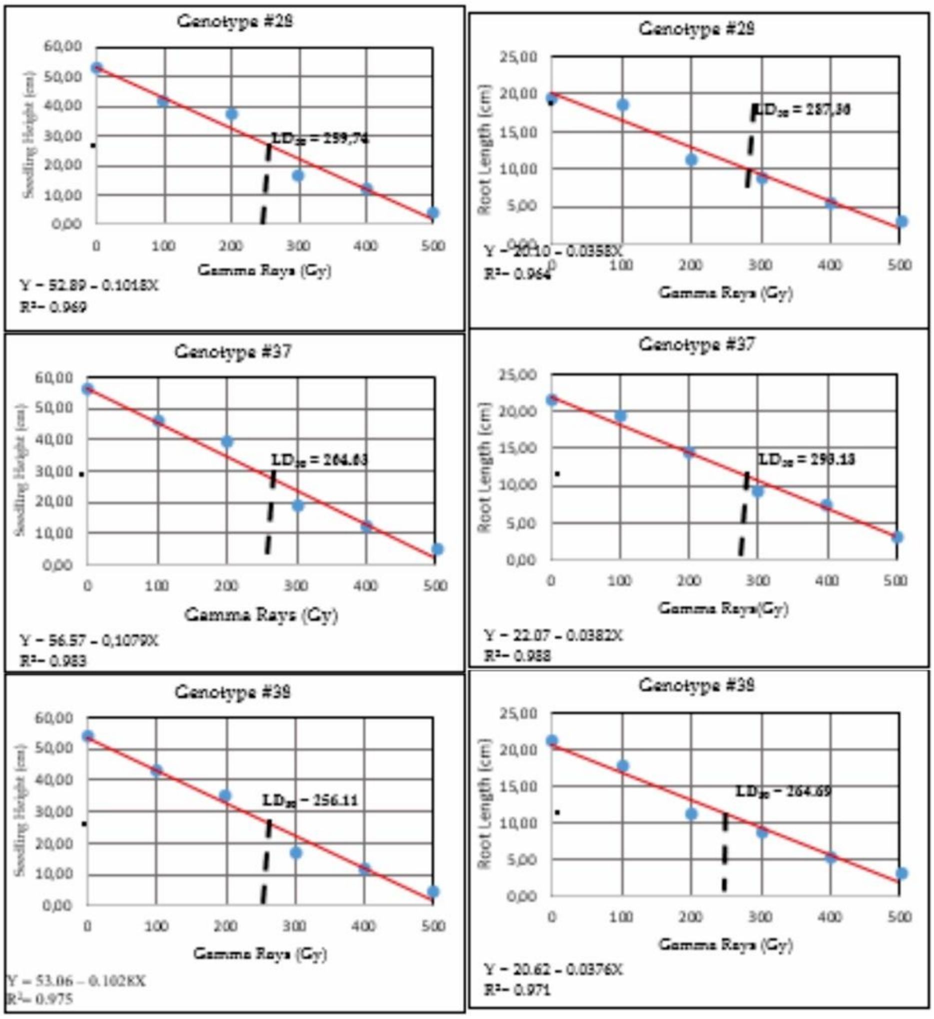

Figure 2. The effect of gamma irradiation on seedling height and root length of three adlay genotypes at 21 days after planting (DAP).

Table 1. LD_{50} and regression on characters of survival, seedling height, and root length three adlay genotypes caused by gamma-ray irradiation.

Characters	Genotypes	N	Regression	R^2	LD_{50}
Survival (%)	#28	1117	$Y = 100.630714 - 0.012678X - 0.000386X^2$	0.948	346.0
	#37	1213	$Y = 94.757857 + 0.077345X - 0.000510X^2$	0.919	381.0
	#38	1197	$Y = 98.862143 + 0.036629X - 0.000452X^2$	0.983	371.0
Seedling height	#28	1117	$Y = 52.89 - 0.1018X$	0.969	259.7
	#37	1213	$Y = 56.57 - 0.1079X$	0.983	264.6
	#38	1197	$Y = 53.06 - 0.1028X$	0.975	256.1
Root length	#28	1117	$Y = 20.10 - 0.0358X$	0.964	287.3
	#37	1213	$Y = 22.07 - 0.0382X$	0.988	293.1
	#38	1197	$Y = 20.62 - 0.0376X$	0.971	264.6

Note: LD = Lethal dose; N = Number of plants; R^2= Determinant coefficient

The LD_{50} values in genotypes #28, #37, and #38 obtained based on seedling height were 260 Gy, 265 Gy, and 256 Gy, respectively, and based on root lengths of 287 Gy, 293 Gy, and 265 Gy (Table 1). Based on regression analysis showed that three adlay genotypes by increasing the doses gamma ray irradiation becomes lower all characters, such as genotype #28, regression value $Y = 100.630714 - 0.012678X - 0.000386X^2$, and all R^2 value > 0.9. This result indicated that in the dosage range, it can reduce seedling height and seedling root length from three adlay genotypes by 50%. The results showed that genotype #37 has a higher LD_{50} value than genotypes #28 and #38, both in the character of seedling height or root length. These results indicated that genotype #37 has the lowest radiosensitivity compared to genotype #28 and #38 against gamma-ray irradiation. This result is similar to research on rice by Warman et al. (2015) who reported that gamma-ray irradiation with doses higher than 200 Gy significantly inhibited seedling growth and root length reduced by 50%. According to Khadimi et al. (2016), gamma rays induce growth inhibition cell cycle during somatic cell division and damage to the entire genome.

Damage to plant cells is caused by exposure to ionize radiation which reacts with water molecules and releases ionization energy to form free radicals (Esnault et al., 2010). These free radicals can damage or modify important plant cell components and influence certain physiological and biochemical processes that are important for the survival of plants. Exposure to gamma-ray irradiation at high doses of seeds interferes with protein synthesis, hormone balance, leaf gas exchange, and enzyme activity and morphological, structural, and functional changes depending on the strength and duration of exposure to gamma-ray doses (Li et al., 2021).

Gamma-ray irradiation treatment also affected the seedling height and root length of the seedling roots. This treatment significantly caused a decrease in the seedling height and root length of adlay seedlings along with increasing doses of gamma-ray irradiation (Figure 3). The decrease in seedling growth due to mutagen treatment is thought to be caused by a decrease in mitotic activity in the meristem tissue (Yadav et al., 2019). Mutagen treatment can cause damage to cell division and cell elongation processes. This is because irradiation causes DNA damage in plant cells which results in various types of damage to plant cell division, plant growth, and development processes (Li et al., 2021). Decreased seedling growth after gamma irradiation treatment also occurred in chili (Sood et al., 2016), okra (Asare et al., 2017), maize (Yadav et al., 2019), and wheat (Kiani et al., 2022).

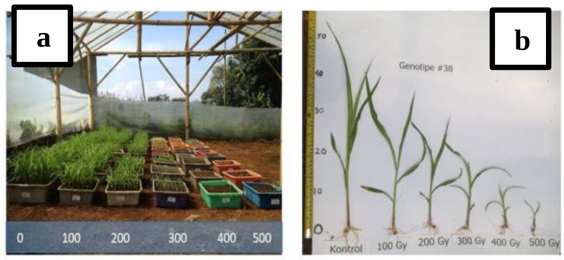

Figure 3. Effect of gamma rays on the seedling growth of three adlay genotypes 21 days DAP, (a) The seedling condition in Plastic house; (b) The different height seedling genotype #38 various doses gamma ray irradiation

Chlorophyll Mutation. There are two kinds of effects caused after gamma irradiation, namely physiological damage and genetic mutation (Qosim, 2020). Physiological damage occurs as a result of mutation effects usually occur in the M_1 generation which is characterized by physical changes in the leaves commonly referred to as chlorophyll change. In this experiment, the types of mutations that appear were albino, albino green, viridis, tigrina, striata, variegata, and maculate (Figure. 4).

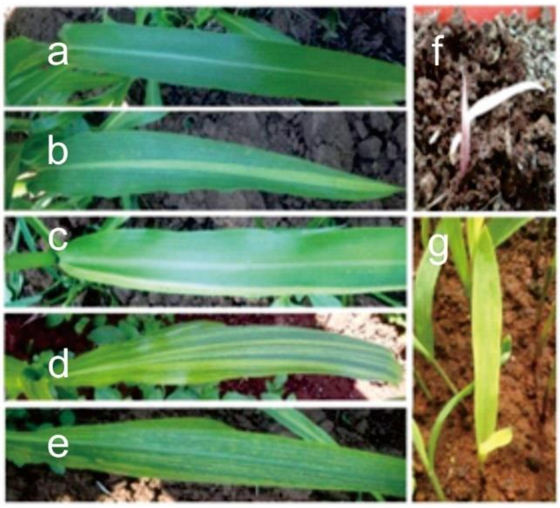

**Figure 4. Chlorophyll change in the adlay a: Control/normal,
b: Sriata, c: Tigrina, d: Variegata. e: Maculata, f: Albino, g: Viridis**

ISSN: 1412-4718, eISSN: 2581-138x

Table 2. Frequency and spectrum of chlorophyll change in M_1 generation of adlay.

Treatment	No. of M_1 plant progenies	Frequency of mutation (%)							TF (%)
		Tig	Str	Vir	Var	Mac	Alb	Albg	
100 Gy	542	0.55	0.92	1.84	0.00	2.76	0.00	0.00	6.07
200 Gy	533	0.56	1.12	1.68	0.56	8.44	0.00	0.00	12.36
300 Gy	329	0.60	0.91	0.60	0.91	18.23	0.00	0.23	21.48
40 Gy	54	3.70	5.55	0.00	0.00	37.03	0.00	0.00	46.28
500 Gy	19	0.00	0.00	0.00	0.00	26.31	5.26	0.00	31.57

Note: Tig (Tigrina), Str (Striata), Vir (Viridis), Var (Variegata), Mac (Maculata), Alb (Albino), Albg (Albino green), TF (Total Frequency).

Adlay plants with the type of albino mutation in this experiment died before growing high because the condition of the plants was weak due to the leaves did not have chlorophyll, grains to carry out photosynthesis as food providers. According to Kolar et al. (2011), plants with the type of mutation xantha and albino generally will die because they do not have chlorophyll and grains so they cannot carry out photosynthesis perfectly. Among the mutants recorded, maculata type was more predominant followed by striata and tigrina.

The results of the observation showed that gamma irradiation treatments with doses of 100 - 500 Gy were able to produce variation in the spectrum and frequency of different chlorophyll change in the M_1 generation (Table 2). In this experiment, the irradiation treatment of 400 Gy dose was able to produce the highest frequency of chlorophyll change with a total frequency of 46.28 %. Meanwhile, the irradiation treatment of 300 Gy was able to produce the broadest chlorophyll change spectrum with 6 types of chlorophyll change consisting of tigrina, striata, viridis, variegata, maculata and albina green. These results indicate that irradiation with these doses is effective in inducing mutations and increasing the diversity of populations M_1 generation in adlay.

The results showed that increasing the dose of gamma irradiation from 100 - 400 Gy showed an increase in the frequency of total chlorophyll change but at a dose of 500 Gy there was a decrease in the frequency of total chlorophyll change. According to Kolar et al. (2011), gamma-ray mutagens can increase the frequency of mutations at low doses and will experience a decrease in the number of mutations at higher doses, namely when the dose has reached the lethal dose point. The results showed that increasing the dose of gamma irradiation from 100 - 400 Gy showed an increase in the frequency of total chlorophyll change but at a dose of 500 Gy there was a decrease in the frequency of total chlorophyll change. According to Kolar *et al*. (2011), gamma-ray mutagens can increase the frequency of mutations at low doses and will experience a decrease in the number of mutations at higher doses, namely when the dose has reached the lethal dose point. The spot and streaks phenomenon that occure on the M_1 leaves might be of a chlorophyll deficiency due to mutations happened in nuclear or cytoplasmic genes (Amirikhah et al., 2019). Chlorophyll change could be as an indicator for evaluating the effect of mutagen treatment (Nilahayati et al., 2016). According to Kolar et al. (2011), chlorophyll change could be of useful for identifying mutagen dose limits that might increase genetic diversity in the M_2. When the levels of radiation increase above the maximum limit of the tolerance of the plants, the capabilities of the photosynthetic apparatus will be decreased by damaging the photosystem, thus resulting in a decrease in chlorophyll content (Perez-Ambrocio et al., 2018; Saha & Paul, 2019). In addition, high-dose irradiation can also indirectly affect photosynthesis by impairing the stomatal conductance and transpiration rate (Saha & Paul, 2019). Amirikhah et al. (2019) reported that low doses of gamma rays (15-krad) significantly increased the total chlorophyll content of tall fescue, but high doses of gamma rays (40-krad) inhibited chlorophyll synthesis of the plants.

Conclusion

Based on the results of the research, it can be concluded that:

1. The LD_{50} value for each genotype is 346 Gy for genotype # 28, 381 Gy for genotype # 37, and 371 Gy for genotype # 38 and the optimum dosage is recommended to induce

mutations in genotypes # 28, # 37, and # 38 namely 300 Gy.

2. The treatment of gamma-ray irradiation at a dose of 100-500 Gy gave different responses to chlorophyll change, the irradiation treatment of 400 Gy dose was able to produce the highest frequency of chlorophyll change with a total frequency of 46.28%. treatment of 300 Gy was able to produce the broadest chlorophyll mutant spectrum with 6 types of chlorophyll change consisted of tigrina, striata, viridis, variegata, maculata and albina green.

Acknowledgments

The authors are grateful to the Academic Leadership Grant (ALG), Universitas Padjadjaran which has funded research activities. We also thanks to the Research Center for Radiation Processing Technology (PRTPR) – National Research and Innovation Agency (BRIN) for facilitating the gamma irradiator facility

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