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The Effects of Gamma Irradiation on Growth Response of Rodent Tuber (*Typhonium flagelliforme* Lodd.) Mutant in *In Vitro* Culture

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Rodent tuber (*Typhonium flagelliforme* Lodd.) is an Indonesian native plant that potential as anticancer. Even though the potential use of bioactive compounds from *T. flagelliforme* is very high, its genetic variation in Indonesia is low. Gamma irradiation can be used to increase genetic variation. The objective of this research was to obtain the first generation mutants (M_1) of *T. flagelliforme* resulting from gamma irradiation of normal mother plantlets with different plant height and number of shoots. *T. flagelliforme* was irradiated at the doses of 0, 10, 20, 30, 40, and 50 Gy. The percentage of plant death was then calculated to determine the Lethal Dose (LD) 50. *T. flagelliforme* was then re-irradiated at LD_{50} dose to obtain M_1 mutant. The plant height and number of shoots variation was observed after eight weeks of culture. The analysis of *T. flagelliforme* radiosensitivity resulted in LD_{50} at 25 Gy. Observation at six weeks after irradiation showed significant differences between the normal mother plant, 20 Gy mutants, and 25 Gy mutants with an average plant height of 9.57, 3.41, and 2.43 cm respectively, and the average number of shoots produced was 7.85, 6.03, and 5.00 shoots respectively. Irradiation at 20 and 25 Gy produced 49 and 37 mutant plantlets. This research showed that gamma irradiation at LD_{50} dose could generate plant height and number of shoots variation of M_1 mutant plantlets that are different from normal mother plantlets.

Keywords: *Typhonium flagelliforme*, irradiated mutant, variation, plant height, number of shoots

INTRODUCTION

Rodent tuber (*Typhonium flagelliforme* Lodd.) is a member of the Araceae family and classified as a medicinal plant. *T. flagelliforme* can be found in several countries such as Indonesia (Syahid & Kristina 2007), India (Rout 2006), Malaysia (Chan *et al*. 2005; Nobakht *et al*. 2009), and other countries in Asia. The availability of *T. flagelliforme* in nature is very limited even though its usage has many benefits. *T. flagelliforme* is very sensitive to specific environmental condition, so the plant tissue culture technique is required to produce a large amount of *T. flagelliforme* plant with the same genetic composition (Nobakht *et al*. 2009).

T. flagelliforme is a herbal plant that has a wide variety of bioactive compounds. Every part of the plant, such as roots, tubers, stems, or leaves, contains anticancer compounds (Choo *et al*. 2001). The bioactive compounds in *T. flagelliforme* were reported as alkaloid, flavonoid (Nobakht *et al*. 2010), saponin (Syahid 2008), sterol (Lai *et al*. 2010), cerebroside, and glycoside (Huang *et al*. 2004). The problems encountered by *T. flagelliforme* is low genetic variation (Nobakht *et al*. 2010), while the potential usage of the bioactive compounds from *T. flagelliforme* is very high. Mutation induction can be used to increase genetic variation in order to obtain plant with higher content of bioactive compounds (Aly 2010).

In vitro culture of Indonesian *T. flagelliforme* has been successfully done (Syahid & Kristina 2007; Sianipar *et al*. 2011). The combination of *in vitro* culture and mutation induction is an effective way to increase variations in plants and produce mutants (Hasbullah *et al*. 2012). Gamma irradiation is most commonly used and most favorable for the induction of mutants (Ahloowalia *et al*. 2004). Gamma irradiation may cause changes in physiology and biochemistry of mutants because it contains a large amount of kinetic energy, causing structural changes

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in the chromosomes of plants (Datta *et al*. 2011). This research reported that gamma-irradiated *T. flagelliforme* producing mutants varied in plant height and number of shoots, which differs from its normal mother type. This study aimed to discover the effect of gamma irradiation on growth response of rodent tuber mutant in *in vitro* culture.

MATERIALS AND METHODS

Plant Material. *T. flagelliforme* plants used in this research were obtained from Aromatics and Medicinal Plant Research Agency (BALITTRO), Bogor, Indonesia. The plants were propagated in the Biology laboratory, Pelita Harapan University, Indonesia, on optimized plant tissue culture medium containing Murashige and Skoog (MS) basal medium, 1 mg/L NAA, 0.5 mg/L BAP, 30 g/L sucrose, 10% coconut water, and 8 g/L agar (Sianipar *et al*. 2011). The plantlets produced were then used in analysis of radiosensitivity to Gamma irradiation.

Analysis of *T. flagelliforme* Radiosensitivity to Gamma Irradiation. Radiosensitivity analysis was performed using Cobalt 60 irradiation (^{60} Co) at the Center for Application of Isotope and Radiation Technology, National Nuclear Energy Agency (BATAN) at doses of 0, 10, 20, 30, 40, and 50 Gy using single dose irradiation method. Each irradiation dose was repeated 12 times. The percentage of death was observed in each irradiation dose.

Determination of Lethal Dose 50 (LD_{50}). Determination of LD_{50} was performed by collecting *T. flagelliforme* survival percentage data on each irradiation treatment and analyzed using Data Fit v.8.2 (Oakdale Engineering) software. A regression equation was then used to determine the LD_{50}.

***T. flagelliforme* Mutation Induction.** Mutation induction was performed by using LD_{50} values previously obtained as a reference to the induction of *T. flagelliforme* mutants. Radiation treatment was performed on a total of 76 plantlets (38 *T. flagelliforme* culture bottles containing two plantlets) at the irradiation dose of around LD_{50} using the single irradiation method. The observation of morphological characters was conducted during the preparation of culture and until six weeks after irradiation. The parameter observed was the average of plant height and number of shoots.

Data Analysis. The data was then subjected to analysis of variance (ANOVA) and Duncan Multiple Range Test (DMRT) using SPSS v. 17 software.

RESULTS

Analysis of *T. flagelliforme* Radiosensitivity. The percentage of plantlets that survived after irradiation decreased with the increase of the given irradiation dose. The data is shown in Figure 1, where the results obtained by treatment with irradiation at the dose of 0, 10, 20, 30, 40, and 50 Gy show the *T. flagelliforme* LD_{50} at 25 Gy. LD_{50} was determined based on the regression equation $y = 0.003x^3 – 0.2296945x^2 + 1.8522x + 99.88769$. There were no deaths at 0 Gy treatment, while treatments with irradiation levels higher than the LD_{50} value caused a high number of plantlet deaths. The irradiation dose above 40 Gy caused the death of all *T. flagelliforme* plantlets.

Mutant Plant Height and Number of Shoots Variation. Figure 2 showed the observed abnormalities caused by gamma irradiation to *T. flagelliforme* plantlets six weeks after irradiation. Plantlet mutants of 20 and 25 Gy had a short height and low number of shoots compared to the normal mother plantlets. Mutation induction to the *T. flagelliforme* plantlets produced 64.47% mutant plantlets at 20 Gy and 48.68% mutant plantlets at 25 Gy (Figure 3). The results of gamma irradiation at 20 and 25 Gy showed significant plant height and number of shoots changes from the normal mother plantlets (Table 1). The observation at six weeks after irradiation showed significant differences between a normal mother plant, a 20 and a 25 Gy mutant, with an average plant height of 9.57, 3.41, and 2.43 cm respectively, and the average number of shoots

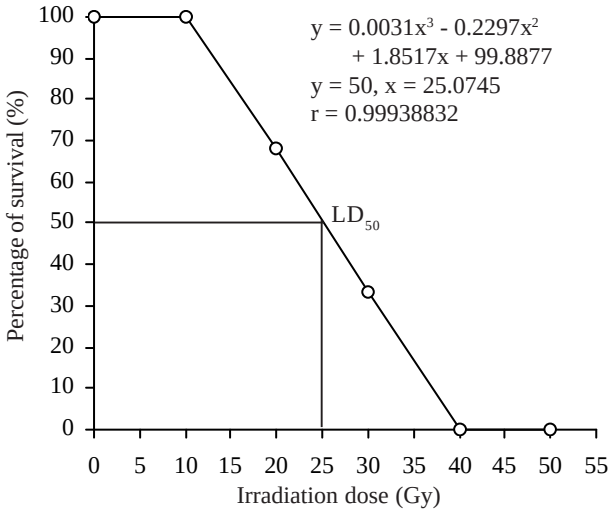

Figure 1. LD_{50} regression equation of gamma irradiation doses on *T. flagelliforme*.

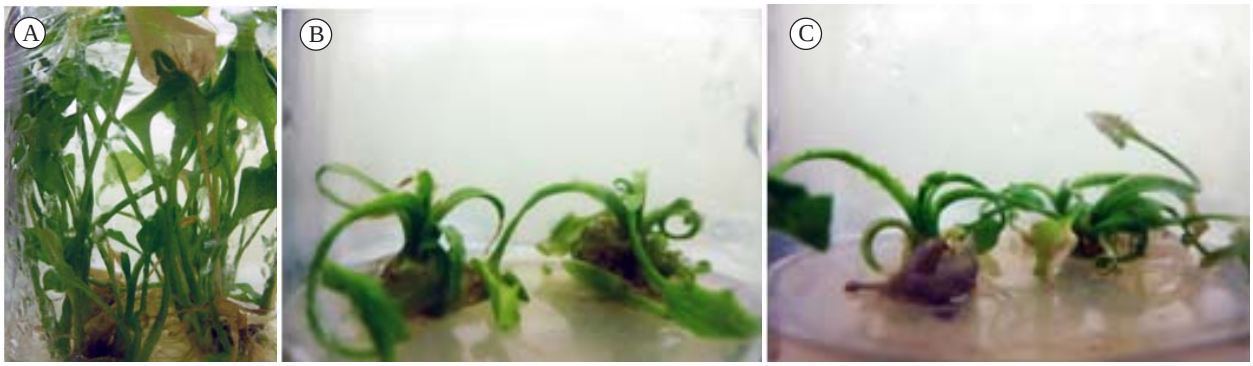

Figure 2. Morphology appearance of *T. flagelliforme* six weeks after irradiation. A. 0 Gy, B. 20 Gy, and C. 25 Gy.

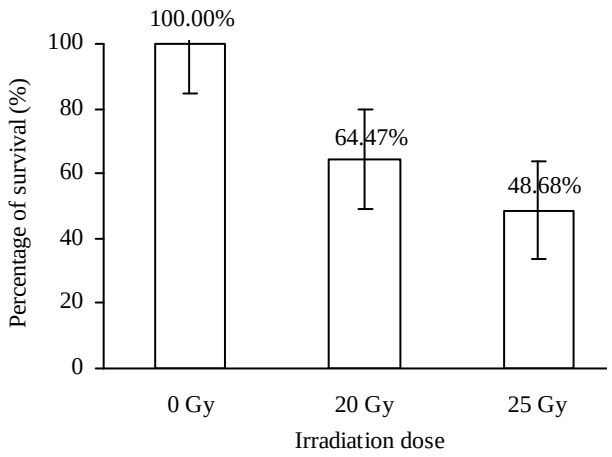

Figure 3. Effect of irradiation dose on the percentage of *T. flagelliforme* survival.

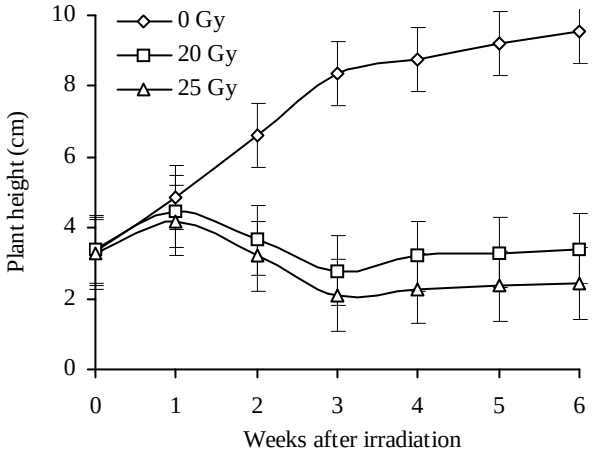

Figure 4. Effect of irradiation dose on the *T. flagelliforme* plant height.

Table 1. Plant height and number of shoots observed at six weeks after irradiation

Dose (Gy)	Number of plantlets (n)	Percentage of survival (%)	Plant height (cm)	Number of shoots (n)
0	16	100.00	9.57[c]	7.85[b]
20	49	64.47	3.41[b]	6.03[a]
25	37	48.68	2.43[a]	5.00[a]

Means of plant height and number of shoots followed by the same letter are significantly different (Duncan multiple range test, P = 0.05)

produced were 7.85, 6.03, and 5.00 shoots respectively. Irradiation at 25 Gy showed the most significant differences compared to the other doses in terms of percentage of survival, average plant height, and average number of shoots.

The development graph of *T. flagelliforme* plant height after gamma irradiation showed a different growth rate between normal mother plantlets and mutant plantlets (Figure 4). In normal mother plantlets, an increase in plant height occurred until the sixth week, while the mutant plantlets had a decrease in plant height from one to three weeks after irradiation followed by a very slow increase until six weeks after irradiation. The higher dose of irradiation given caused a decrease of the plant height. These results indicated that the irradiation dose given to the plant significantly affected the plant height.

The development graph of *T. flagelliforme* shoots after gamma irradiation showed a different growth rate of the number of shoots between normal mother plantlets and mutant plantlets (Figure 5). The number of shoots tended to increase from one week after irradiation, but, six weeks after irradiation, the number of shoots produced was different. The normal mother plants produced the highest amount of shoots followed by the 20 and 25 Gy mutants respectively. The higher dose of irradiation given caused a significant decrease of the number of shoots produced compared with the normal mother plants, but the 20 and 25 Gy irradiation doses made no significant difference on the mutants' number of shoots. These results indicated that the irradiation dose given to plants have a significant effect on the number of shoots produced.

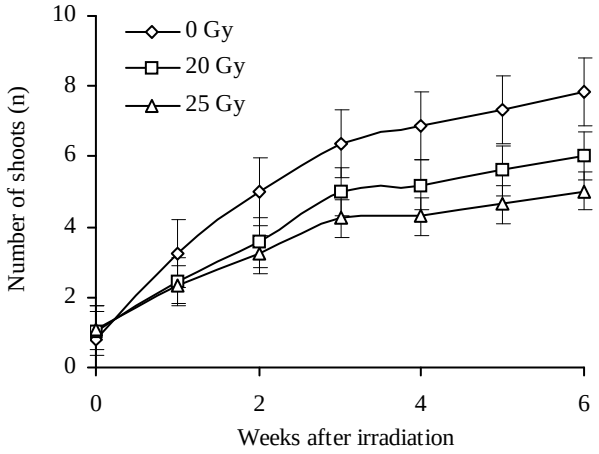

Figure 5. Effect of irradiation dose on the *T. flagelliforme* shoot number.

DISCUSSION

Gamma irradiation can be used to induce mutation and increase genetic variation. Gene mutations can lead to the emergence of different phenotypes from mother plants and can be inherited (Encheva 2009; Morad *et al*. 2011). The higher the dose given, the greater are the chances of mutation. An effective dose is a dose which causes only 50% mortality of the plantlets treated with mutagen (Herison *et al*. 2008). Parts of the plants that are actively dividing, such as the meristem, are highly sensitive to irradiation. The main targets of irradiation are genes. Irradiation ionizes the atoms in tissues by removing electrons from atoms. Ionization damages the bases of DNA and causes them to be mismatched. Chromosome strands can be broken by irradiation and can change the structure of the chromosome in four ways through deletion, inversion, duplication, and translocation. The damage to the molecular level causes the increase of variation in irradiated plants (van Harten 1998).

In this research, irradiation was performed at the dose of 10- 50 Gy, and the radiosensitivity curve showed the *T. flagelliforme* LD_{50} at 25 Gy. On other plants, a different irradiation dose was given to obtain mutants, depending on the species of the plant. Irradiation to produce mutants of *Mangifera indica* was conducted at the doses of 10-50 Gy (Van Harten 1998), *Triticum durum* at the doses of 10-30 Gy (Melki & Dahmani 2009), *Curcuma alismatifolia* at the doses of 10-100 Gy (Abdullah *et al*. 2009), *Gerbera jamesonii* at the doses of 10-60 Gy (Hasbullah *et al*. 2012), and *Hordeum vulgare* at the doses of 10-30 Gy (Khan *et al*. 2003). This research showed that *T. flagelliforme* has a different radiosensitivity than other plants.

Re-irradiation was performed on 76 *T. flagelliforme* plantlets to observe the changes in the plant height and the number of shoots that occurred due to irradiation at a dose of around LD_{50}. Then, based on a radiosensitivity analysis, the irradiation was performed at 20 and 25 Gy to effectively induce mutations in *T. flagelliforme* plantlets. The percentage of the plantlets that survived at 20 and 25 Gy was 64.47% and 48.68% respectively. This showed that the mutation induction performed has produced mutants that can survive irradiation. Mutation induction at LD_{50} dosage (25 Gy) resulted in a percentage of death around 50%. This data confirmed the radiosensitivity analysis which produced LD_{50} at 25 Gy.

Analysis of radiosensitivity and mutant morphology variation caused by gamma irradiation has never been done before on *T. flagelliforme*. Several other researches have shown changes in the morphological characters of plants treated with irradiation. Morphological characters variation due to irradiation occurred in *Stenotaphrum secundatum* (Li *et al*. 2010), *Helianthus annuus* (Jagadeesan *et al*. 2008), *Stylosanthes guianensis* (Tan *et al*. 2009), and *Vigna unguiculata* (Adekola & Oluleye 2007). *In vitro* plants treated by mutagen produced an increasing frequency of spontaneous mutations that can lead to new mutant cultivar with a desirable trait (Jain 2012). Mutagen treatment on shoots or another plant organ culture causes the formation of chimera that requires further propagation to produce a solid mutant (Datta *et al*. 2005; Misra & Datta 2007).

Significant changes in *T. flagelliforme* plant height and number of shoots compared to normal mother plant showed that the gamma irradiation given to plants has produced *T. flagelliforme* mutants. The plant height and number of shoots formed are strongly dependent on the presence of auxin and cytokine (Chan *et al*. 2000; Syahid & Kristina 2007; Nobakht *et al*. 2009; Sianipar *et al*. 2011). Even though the optimized medium was used to maintain the *T. flagelliforme* mutants in this research, the observed plant height and number of shoots still showed significant changes from normal mother plantlets. Irradiation treatment is strongly suspected to be the cause of significant changes in *T. flagelliforme* plant height and number of shoots compared to normal mother plant.

In conclusion, the analysis of *T. flagelliforme* radiosensitivity resulted in LD_{50} at 25 Gy. Irradiation at the 20 and 25 Gy dose has produced mutants with significant differences in the percentage of survival, the average plant height, and the average number of shoots compared to normal mother plantlets. Different responses at different doses showed that the variation of the irradiation dose caused the variation in the plant height and the number of shoots. This research showed that gamma irradiation at the 20 and 25 Gy dose can generate *T. flagelliforme* M_1 mutant plantlets with significant differences of plant height and number of shoots from normal mother plantlets. Even though there is still a chance the mutant will become a chimera after irradiation, further molecular analysis, such as RAPD, AFLP, and SSR, is still needed to differentiate between a chimera and a potential mutant.

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