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THE EFFECTS OF GAMMA IRRADIATION AND SOMACLONAL VARIATION ON MORPHOLOGY VARIATION OF MUTANT RODENT TUBER (*Typhonium flagelliforme* Lodd.) LINES

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ABSTRACT

Rodent tuber (*Typhonium flagelliforme* Lodd.) is an Indonesian plant with high medicinal potential as anti-cancer. This plant has a low genetic variation in Indonesia. Gamma irradiation can be used to increase genetic variation. This research aimed to explore the effects of gamma irradiation and somaclonal variation on several mutant rodent tuber lines. Six somatic cell populations, which were treated by 6 Gy of gamma irradiation, were successfully regenerated into plantlets. Six mutant lines had been sub cultured into second and third generations by using optimal regeneration media (MS0 supplemented with 0.5 mg/L BAP and 1 mg/L NAA). Rooting of *in vitro* plantlets had been done by using optimal rooting media MS0 supplemented with 0 mg/L NAA and 0.5 mg/L NAA). Plantlets with good roots were acclimatized and transferred into greenhouse. The morphology of first generation mutant in greenhouse (M1) were characterized and analyzed by using descriptive statistical method. The observed morphological characters including plant height, shoot number, leaf number, and leaf area. A hundred and seventy five (175) mutant lines were obtained on the third generation (MV3) from five somatic cell populations. Mutant line 6-3-x has the highest mean number of shoots, which was 4.62 shoots. Percentage of plant alive after acclimatization in the greenhouse was 78%. Out of 37 M1 mutant lines, morphologically diverse lines were observed with the highest plant height increase: 15.5 cm on 6-2-4-1 mutant line, the highest plant shoots increase: 5 shoots on 6-6-7-8 mutant line, the highest leaf number increase: 17 leaves on 6-6-7-8 mutant line, and the highest leaf area increase: 47.24 cm^2 on 6-2-5-2 mutant line. In conclusion, gamma irradiation and somaclonal variation could increase genetic variation of mutant rodent tuber lines as shown by morphological data.

Keywords : *Thyphonium flagelliforme* , Gamma-Irradiation Somaclonal-Variation, Morphology-Variation

INTRODUCTION

Rodent tuber is an Indonesian plant commonly found in Java island and grows well at 1-300 m altitude above the sea (Essai, 1985). Rodent tuber is a medicinal plant. Several countries in Asia has been using the plant to treat cancer (Chan *et al.*, 2005), such as liver cancer (Lai *et al.*, 2008), breast cancer, intestinal cancer, prostate cancer, cervix cancer (Syahid, 2008), and leukemia (Mohan *et al.*, 2010). Sianipar, Maarisit and Valencia (2013) had reported the toxicity effect rodent tuber's extract and hexane fraction on *Artemia salina*.

Rodent tuber has low genetic variation because the plant commonly propagates vegetatively through tuber separation (Syahid & Kristina, 2007). Gamma irradiation is one of methods to increase genetic variation. Gamma irradiation is one of the most commonly used mutation breeding method (Natawijaya *et al.*, 2009) because it could change the chemical reaction in plant cells and could cause chromosomal aberration in plants (Achrom *et al.*,

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2011). Genetical changes caused by mutation can also cause changes in secondary metabolites produced by plants (Kovács & Keresztes, 2002). Mutated genes will change and produce different variation of proteins or enzymes (Karp, 2008).

Rodent tuber had been successfully propagated through in vitro techniques in Murashige Skoog (MS) medium with optimal concentration of NAA 1 mg/l and BAP 0.5 mg/L (Sianipar *et al*., 2011). In vitro rodent tuber plants had been successfully irradiated with gamma irradiation and mutant rodent tuber plantlets had been obtained. Mutant in vitro rodent tubers showed difference in height and number of shoots compared to control plantet (Sianipar *et al*., 2013). Genetic variation selection of first generation field mutant rodent tuber (M1) should be done to screen mutants with high genetic variation. This research aimed to know the effect of gamma irradiation and somaclonal variation to morphological variation of several different lines of mutant rodent tubers.

MATERIAL AND METHODS

Propagation of Mutant Plants

Eight weeks old *in vitro* mutant plantlets of rodent tuber were subcultured to optimal propagation medium, which is Murashige Skoog (MS) medium supplemented with 1 mg/l NAA and 0.5 mg/L BAP. After ten weeks of *in vitro* culture, mutant plantlets were acclimated in acclimatization medium of sekam : compost (1:1) for four weeks and then moved to greenhouse.

Rooting of Mutant Rodent Tuber Plantlets

Rooting of mutant rodent tuber plantlets was done in MS0 medium supplemented with coconut water, increasing NAA concentration (0; 0.5; 1; 1.5 mg/l) and combination 0.5 mg/l BAP with increasing NAA concentration (1 and 1.5 mg/l). Rooting percentage of the plantlets was observed in the eight week of culture.

Acclimatization of Mutant Rodent Tuber Plantlet

Acclimatization of mutant rodent tuber plantlets was done in Laboratory of Advance Biology Universitas Pelita Harapan. Mutant plantlets were taken out of culture bottles, washed with flowing water and sunk in bacteriside fungiside (0.25 gram each in 200 ml of water) solution for 15 minutes. Plantlets were plantlet into medium containing sekam and compost. Viability percentage of plantlets was observed for four weeks. Living plantlets were then moved to greenhouse at BB-Biogen and plantler in soil medium. Viability percentage of the acclimated plantlets was observed for four weeks.

Morphological Characterization of Mutant Rodent Tuber

The morphological characters of mutant rodent tuber observed were leaf surfaces, plant height measured from above the soil to the highest leaf, the number of leafs per plant, and the number of shoots per plants. Morphological characters were observed every two weeks for eight weeks.

RESULT AND DISCUSSION

Propagation of Mutant Plants *In Vitro* Rodent Tuber

Callus of rodent tuber were irradiated with gamma light at 6 gy dose. The *in vitro* mutant calli were propagated and regenerated in MS medium with 1 mg/l NAA and 0.5 mg/l BAP. This medium was the optimal medium for propagation of in vitro rodent tuber Bogor cultivar (Sianipar *et al*., 2013). Second and third generation mutant were obtained through *in vitro* culture in optimal medium. Mean number of shoots of third generation mutant rodent tuber obtained from *in vitro* culture were shown for each line in Table 1.

Table 1. Regeneration of Third Generation Mutant *In Vitro* Rodent Tuber

Klon	Replikasi (n)	Rataan	StDev	Mean±StDev	Std Error
6-1-x	8	3,25	1,28	3,25 ± 1,28	0,45
6-2-x	10	4,30	3,30	4,30 ± 3,30	1,04
6-3-x	8	4,62	3,81	4,62 ± 3,81	1,34
6-6-x	16	4,31	3,68	4,31 ± 3,68	0,92
6-7-x	3	4,00	4,35	4,00 ± 4,35	2,51

Compared to the other mutant lines, mutant rodent tuber M6/3-x line showed the highest mean number of shoots, which was 4.62 shoots. Mutant line M6/7-x showed the lowest mean number of shoots, which was 3.25 shoots. Each line had different variation of shoots number as shown in standard deviation (Table 1). After ten weeks of culture, *in vitro* plantlets were sub cultured into new medium through tuber separation (Figure 1).

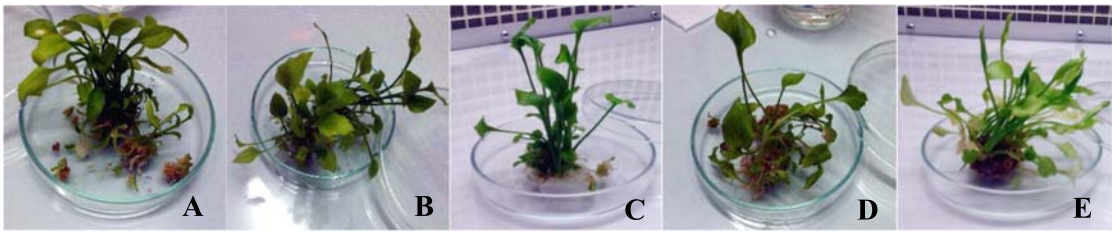

Figure 1. Subculture of Third Generation Mutant Rodent Tuber
A: Mutan- 6/1-4-2; B: Mutan-6/2-6-3; C: Mutan-6/3-3-3; D: Mutan-6/6-1-6; E: Mutan-6/7-1-6

In vitro irradiated calli of rodent tuber showed the ability to regenerage and form plantlets up to the third generation. Number of shoots obtained showed the ability of the mutant plantlets to survive and reproduce vegetatively. Gamma irradiation in 5-15 gy dose in nilam plant affected positively on the growth of nilan plant (Kadir, 2011a) Gamma irradiation of ginger on 7.5 and 12.5 gy could induce the growth of leafs, shoots, and roots of ginger plant (Devy & Sastra, 2006). The plant tissue of *Anthurium* which was irradiated by gamma light on 5 gy dose showed good response in regeneration and callus induction (Puchooan & Sookun, 2003). The use of mutagen at high dose could give several somatic effects on mutated plants (Pucchoa & Sookun, 2003). Gamma irradiated rice mutant showed decrease in shoots number after the addition of polyethylene glycol (PEG) (Kadir, 2011b).

Media Optimation and rooting induction Mutant Rodent Tuber Plantlet

In vitro rooting had been done with six treatments of growth regulators (Figure 2). Four out of six treatments used were able to induce roots in mutant rodent tubers, which were 0 mg/l

NAA, 0.5 mg/l NAA, 0.5 mg/l BAP + 1 mg/l NAA, and 0.5 mg/l BAP + 1/5 mg/l NAA.

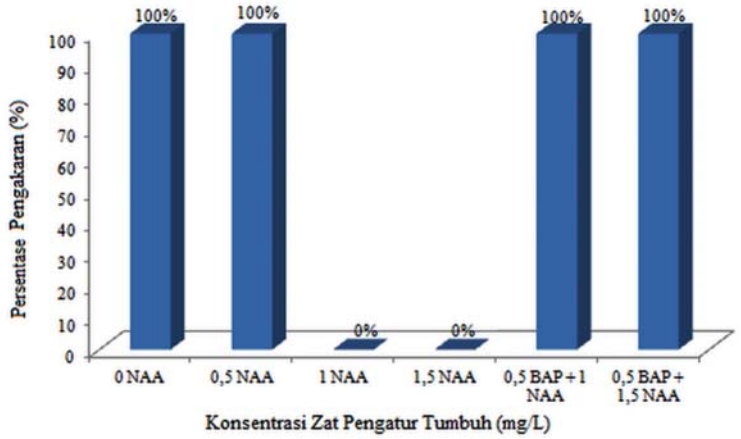

Figure 2. Rooting Percentage of Rodent Tuber Mutant with NAA and BAP

The growth regulator auxin had been known to have several effects on plant's root, which were elongation of root, proliferation of root cells, and differentiation of root cells at certain concentration. High concentration of auxin might inhibit the elongation of root cells (Teale *et al.*, 2005). Rooting data showed decrease in rooting percentage as opposed to the increase of NAA concentration (Figure 2). This data indicated high concentration of endogenous auxin in mutant rodent tubers.

Ratio of auxin and cytokinin had been known to affect the morphogenesis of root in tissue culture (Teale *et al.*, 2005). The use of NAA and BA combination in this research was more effective than high concentration of NAA alone (Figure 2). Combination of growth regulator BAP and IBA, and the addition of charcoal had also been proven effective in rotting of *Dendrobium* plant (Khatun *et al.*, 2010).

Acclimatization of mutant rodent tuber plantlets

Acclimatization of mutant rodent tuber plantlets was done for four weeks. Total clones of mutant acclimated were 95 clones. Viability percentage of acclimatization was 66% on week four (Figure 3).

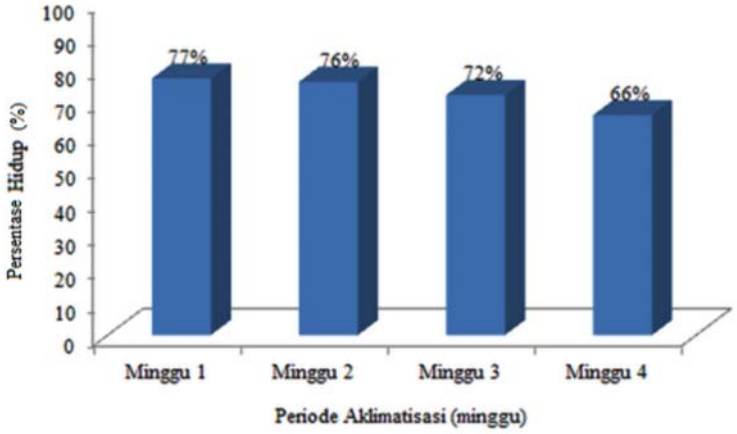

Figure 3. Viability Percentage of Mutant Rodent Tuber Plantlets

Figure 3 showed the development of mutant rodent tuber during acclimatization period. Several shoots of mutant rodent tuber died during the first week of acclimatization. Some

others survived up to week four.

Acclimatization of in vitro plantlets was affected by several factors, such as acclimatization media (Mishra & Ansari, 2011), light intensity, and moisture (Hazarika, 2003). The viability percentage obtained in this research (66%) was lower than viability percentage of Malaysian rodent tuber, which was 90% (Nobakht, Kadir & Stanslas, 2009). This difference was probably due to two factors. The first factor was genetic changes of mutant rodent tuber. Genetic changes that had occurred in mutant rodent tuber might render mutant rodent tuber vulnerable to ex vitro conditions. The second was the difference in acclimatization media used in the experiment. Nobakht *et al.* (2009) used peatmoss:perlite:vermiculite (3:1:1) media for acclimatization.

Acclimated plantlets were moved to greenhouse after acclimatization. Fourty seven clones were observed for post-acclimatization observation in the greenhouse. The viability percentage obtained post-acclimamtization was 78% (Figure 4 and Figure 5). Acclimatization in general has successfully removed the weaknesses of plantlets due to in vitro culture condition, such as dependence to high moisture, vulnerability to high light intensity, and vulnerability to microbes (Posposilova, 1999).

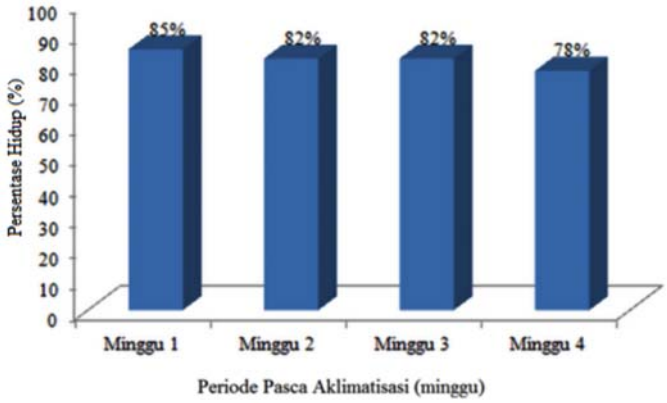

Figure 4. Post-acclimatization viability percentage of mutant rodent tuber

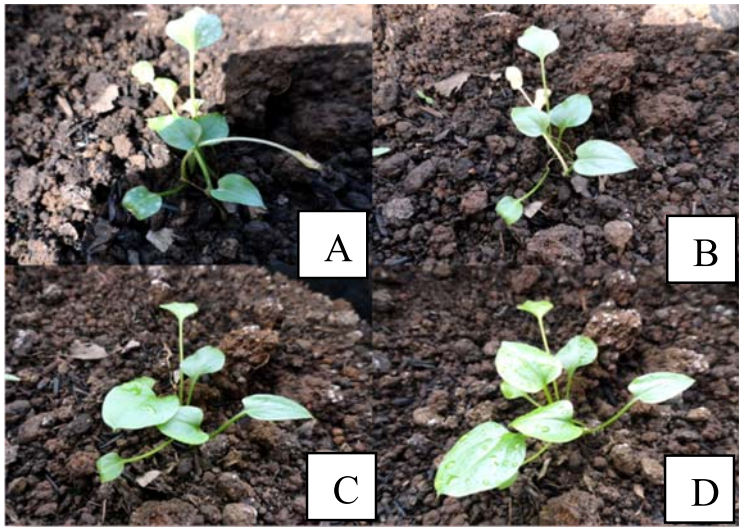

Figure 5. Post-acclimatization mutant rodent tuber plant
Plant age (A) 1 week, (B) 2 week, (C) 3 week, (D) 4 week

Morphological characterization on Rodent Tuber Mutan plantet

Morphological characterss of rodent tuber mutants had been observed in greenhouse of BB-Biogen. Characterization was done to 37 mutant lines of rodent tuber. Morphological characters obtained in general were variable (Table 2). High standard deviation observed between mutant lines in comparison to control. Mutation through gamma irradiation and somaclonal variation had successfully cause higher variation in morphology of mutant rodent tuber lines. Gamma irradiation caused changes in genome, chromosome, gene, and organelle (Qosim, 2006)

The highest increase in leaf area and leaf circumference was observed in mutant line 6-9-7, which were 47.41 cm^2 and 15.25 cm. The highest plant height increase was observed in mutant lines 6-2-4-1 and 6-9-3 which was 15.50 cm. The highest increase in number of shoots was observed in mutant line 6-6-7-8 with 5 shoots increase. The highest increase in number of leaves was 17 leaves, observed in mutant line 6-6-7-8.

Morphological characters observed in this research were significance agronomically. Leaf area is important to photosynthesis (Stampar *et al.*, 1999), plant height is important for light exposure (Aarsen, 1995), number of leaves and shoots are linked to biomass of plant. Morphologically selected mutants should have good agronomy traits.

Morphological traits of mutant rodent tuber lines had shown the uniqueness of each mutant lines. Mutant lines 6-1-1-1 for example had high increase in all of the parameters observed. Mutant lines 6-6-7-8 had high increase in number of leaves, but the leaf area and circumference were small. Mutant line 6-3-3-10 had low increase in shoot number, but high increase on the leaf area and circumference. Mutant line 6-3-3-5 had low increase in all of the observed parameters.

The uniqueness of each mutant lines (Table 2) indicated a random fashion of genetic changes, which were most likely due to gamma irradiation and somaclonal variation. The random changes in the genome because of gamma irradiation had also been shown in coconut mutants (Rohani *et al.*, 2012). Several other researches had shown changes in morphological characters due to gamma irradiation, for example in mutant sugarcane (Khan *et al.*, 2000), *Artemisia* (Purnamaningsih *et al.*, 2011), and *Phalaenopsis amabilis* (L.) Bl. (Sulistianingsih *et al.*, 2006).

CONCLUSION

In conclusion, *in vitro* rodent tuber mutant M6/3-x had the highest mean number of shoots, which were 4,62 shoots. Acclimatization of *in vitro* rodent tuber plantlets was successfully done to 95 clones with 66% acclimatization viability percentage. Morphological characters obtained were variable, based on leaf area increase, leaf circumference increase, plant height increase, number of leaves, and number of shoots data. Mutant line 6-1-1-1 was a superior mutant based on the observed morphological characters.

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Table 2. Morphological Characters of Mutant Rodent Tubers

No	Mutant Lines	ΔLeaf Area(cm^2)	ΔLeaf Circumferece(cm)	Δ Plant Height (cm)	Δ Number of Shoots	Δ Number of Leaves
1	6-3-3-6	17,08	8,64	12,80	3	8
2	6-2-8-1	8,01	7,54	8,00	0	5
3	6-6-7-8	5,34	4,40	6,00	5	17
4	6-6-1-9	25,18	11,15	14,00	2	15
5	6-2-5-2	43,35	14,61	12,50	0	5
6	6-9-4	17,67	7,07	9,20	1	5
7	6-2-3	19,44	7,54	12,50	1	9
8	6-9-3	44,00	12,25	15,50	1	7
9	6-9-7	47,41	15,24	14,00	2	9
10	6-9-5	3,22	0,31	4,50	0	1
11	6-2-6(3)	17,40	6,75	11,00	0	4
12	6-1-6-1	2,145	1,41	3,50	1	4
13	6-9-1	40,26	10,37	12,00	3	13
14	6-2-1	15,32	11,00	6,50	0	6
15	6-3-2-1	36,73	17,28	11,40	0	9
16	6-2-8-2	14,96	6,75	11,50	1	2
17	6-2-2-4	3,22	3,61	0,50	0	3
18	6-6-3-6	19,41	5,97	11,50	0	8
19	6-2-4-1	26,47	11,31	15,50	0	1
20	6-3-2-3	9,42	11,00	7,00	3	10
21	6-1-2	38,10	9,58	11,10	2	6
22	6-1-2	26,32	7,85	10,50	3	7
23	6-1-3-3	11,98	6,28	6,50	4	16
24	6-2-2-8	17,00	9,11	8,50	0	3
25	6-2-1-2	13,51	6,75	9,00	1	10
26	6-3-3-5	5,50	4,71	2,50	0	3
27	6-1-1-1	22,07	8,95	11,50	4	11
28	6-1-1-4	6,96	6,28	5,00	0	2
29	6-1-3-4	21,60	8,64	10,20	0	9
30	6-1-1-2	28,91	9,74	10,60	1	5
31	6-1-1-9	23,76	10,68	13,00	2	8
32	6-2-6-2	16,26	7,85	9,10	0	3
33	6-2-13	11,98	7,85	8,50	2	10
34	6-3-3-10	26,40	11,00	12,80	0	1
35	6-6-3-4	3,14	3,45	1,70	0	0
36	6-6-3-7	3,14	6,28	2,20	1	3
37	6-1-1-6	16,50	10,21	10,50	1	9
	Mutant Mean	19,17 ± 12,57	8,36 ± 3,55	9,25 ± 3,94	1,18 ± 1,39	6,67 ± 4,30
	Control Mean	10,03 ± 8,07	3,55 ± 3,20	7,4 ± 1,92	0,00 ± 0,00	1,60 ± 2,07

Mean of control was obtained from 5 replication. Pertambahan variabel dihitung dengan mengurangi nilai variabel pada pengamatan keempat (minggu 8) dan pengamatan pertama (minggu 1). Ä sign on the table showed the increase of each parameter. The increases of each parameter were calculated by substracting data of the eight week of observation and the first week of observation.

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