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In Vitro Mutagenesis of *Etlingera elatior* by Gamma Ray Intermittent Irradiation

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Abstract

Etlingera elatior is tropical ornamental plant commonly called "torch ginger" from Zingiberaceae family. Conventional breeding of *E. elatior* is limited by cross incompatibility, poor fruit set and low seed production. In this study irradiation of *E. elatior* with gamma ray performed to induce mutation. This study was aimed to increase morphological diversity and to obtain unique morphological characters to increase the aesthetic value of *E. elatior* as ornamental plants and cut flower. Two genotypes of *E. elatior*, red and white flowers, were tested. The LD_{20}, LD_{35}, and LD_{50} were determined following intermittent (split dose) gamma irradiation with a two-hour gap between each gamma ray shot. Red genotype *E. elatior* explants were irradiated with dose of 3 + 3 Gy (LD_{20}); 4 + 4 Gy (LD_{35}) and 5 + 5 Gy (LD_{50}); white genotype were irradiated with a dose of 2 + 2 Gy (LD_{20}); 2.8 + 2.8 Gy (LD_{35}) and 3.7 + 3.7 Gy (LD_{50}). Non-irradiated explants were set as control. The results of this study indicated that the increase in dose of gamma ray irradiation changed shoot length and number of leaves in the red genotype per explant as compared to control. Morphological changes occurred in leaf shape at 5 + 5 Gy and 3.7 + 3.7 Gy and formation of variegated leaves at 2.8 + 2.8 Gy and 5 + 5 Gy.

Keywords: mutation breeding, ornamental plant, split irradiation dose, torch ginger, Zingiberaceae

Introduction

Zingiberaceae is the largest family of the order Zingiberales. Many species from Zingiberaceae family are economically important as source of food, spices, medicine and ornamentals. *Etlingera elatior* (Jack) R.M.Sm is known as *kecombrang* or *honje* in Indonesian. *E. elatior* flowers, fruits and seeds have been used as food ingredients in Asia, including Indonesia. *E. elatior* flowers have an attractive colour with varying shades of pink. The red shade bracts and flowers make this species very attractive plant for ornamental purposes (Figure 1) such as for decoration in hotels and offices.

Etlingera flowers have a short vase life; most flowers only last for six to seven days (Sabu et al., 2013). Cut flowers from a tropical country get less attention than flowers originated from sub-tropical countries like roses, carnations and gerberas. Research and breeding of tropical flowers including *E. elatior* is very limited and most studies focus on the phytochemical content of rhizomes for medicinal rather than for ornamental purposes. Chan et al. (2011) reported that phytochemicals from *E. elatior* has antioxidant, antibacterial, antifungal, cytotoxic, hepatoprotective and tyrosinase inhibitor activity.

Figure 1. Morphology of *Etlingera elatior* flowers (a) white (Anderson, 1994) (b) red

Despite having attractive shape and colours *E. elatior* flowers are large and heavy, making, the cost of air transportation expensive (Dwiatmini et al., 2009). *E. elatior* can be a potential cut flower but it requires continuous improvement in flower colour, morphology, longevity, size, odour and reduced time to flower. Unfortunately, conventional breeding of *E. elatior* is limited by cross-incompatibility, poor fruit set and low seed production, therefore gamma ray induction mutation is performed to increase morphological diversity.

Mutation is one of sources of genetic variation. Natural mutations are rarely occurring so to increase the frequency of occurrence of natural mutations induction mutations has been performed using mutagens (Aisyah et al., 2009). Among the physical

mutagens, χ - and γ (gamma) rays are the most commonly used mutagens in mutation breeding. Gamma rays pass through a tissue by ionizing process (Mba and Shu, 2011). Gamma ray irradiation can damage DNA molecules such as the double strand damage, loss of nitrogen bases and changes in chemical base structure that can cause gene mutations. The existence of gene mutations in plant can cause changes in phenotypes which inherited to their offspring called mutant (Van Harten, 2002). Putative mutants are plants which are considered as mutants that expected in next generation to be genetically proven. *In vitro* culture methods have facilitated the use of mutation-assisted breeding technique for improvement vegetative propagated crops (Bala and Kanwar, 2015).

The success of irradiation to increase population diversity is largely determined by irradiated radio sensitivity of the genotype. Radio sensitivity can be measured based on the value of LD_{50} (Lethal Dose 50) which is the dose that causes 50% mortality of the plant population. The optimum dose that can produce the most mutants usually occurs around LD_{50}. LD_{25} - LD_{50} is the dose that can produce the highest mutation rate. Research conducted by Yunus et al. (2013) reported that LD_{50} in shoots *in vitro* is 10 Gy. Based on LD_{50}, LD_{35} and LD_{20} have been obtained in the preliminary study using dose range of 5-25 Gy. In this study, gamma ray intermittent irradiation treatment used in red and white genotype *E. elatior*. Induction of gamma ray intermittent irradiation mutation is expected to increase genetic diversity and can be obtained unique properties that can increase the aesthetic value of ornamental plants and flowers.

Materials and Methods

Plant Materials

This preparation and propagation of plant materials was conducted in the Tissue Culture Laboratory 3, Department of Agronomy and Horticulture, Faculty of Agriculture, Bogor Agricultural University in February to August 2017. The explants were collected from fourteen-month-old *E. elatior* plants with red and white flowers from Mandiri Jaya Flora Nursery, Megamendung, West Java, Indonesia. Individual shoots were excised and cultured on MS medium (Murashige and Skoog, 1962) containing 3 % sucrose (w/v), 0.7 % gel, 31.08 µM BAP. pH medium was adjusted to 5.8 before autoclaving at 121°C for 15 min. The cultures were incubated for six weeks at 23 ± 1°C under 24 hours light with intensity 1200-1500 lux prior to irradiation treatment.

Gamma Ray Irradiation

The irradiation process was carried out at PAIR BATAN, South Jakarta. *E. elatior* plantlets (in tissue culture flasks) of 15 to 30 mm shoot length were irradiated with the ^{60}Co radioisotope source (Gamma Chamber 4000A; dose rate of 288.55 Gy/hr). Irradiation was conducted intermittently with a split dose of LD_{20}, LD_{35}, and LD_{50} with a gap of two hours between irradiation. The values of LD_{20}, LD_{35} and LD_{50} had been determined for both *E. elatior* genotypes in our previous study (unpublished) i.e. 6 Gy, 8 Gy and 10 Gy, respectively, for the red flower genotype, and 4 Gy, 5.6 Gy and 7.4 Gy, respectively, for the white flower genotype. In the current study, red genotype plantlets were irradiated at 0 Gy (control); 3 + 3 Gy (P1); 4 + 4 Gy (P2) and 5 + 5 Gy (P3) whereas the white genotype plantlets were irradiated at 0 Gy (control); 2 + 2 Gy (P1); 2.8 + 2.8 Gy (P2) and 3.7 + 3.7 Gy (P3). There were three replications per dose, and each replication consisted of 10 explants.

Post Irradiation Handling of the Explants

The irradiated explants are designated as MV0. After irradiation treatment, MV0 explants were transferred to a MS medium supplemented with 31.08 µM BAP for shoot multiplication; the new shoots grew from MV0 were designated as MV1. When the MV1 shoots were 15 to 30 mm in length they were excised from the irradiated explants (MV0) and sub-cultured to a new medium with the same hormone composition (MS + 31.08 µM BAP).

Sub-culture was conducted at five weeks intervals for shoot multiplication up to the second regeneration cycle (MV2). Cultures were maintained on the same type of medium by regular sub-culturing every five weeks over a period of 10 weeks. Plant cultures were incubated at 23 ± 1°C with 24-h light photoperiod with light intensity 1200-1500 lux. A radio sensitivity test was determined by measuring the percentage of plantlet survival at five and ten weeks after irradiation. Quantitative characters were measured on the number of leaves per explant, number of shoots per explant and shoot maximum height. Colour and shape of leaves were recorded visually after ten weeks of culture.

Data Analysis

The data from MV1 and MV2 were analysed using boxplot with Microsoft Excel Professional Plus 2016 to show the data characteristics and distribution. Leaf colours were characterized using the Royal Horticultural Society Mini Colour Chart as a reference.

Result and Discussion

The increasing dosage of irradiation decreased the percentage of surviving plantlets at five and ten weeks after irradiation (Table 1).

The number of shoot, shoot length and number of leaves per explant in *E. elatior* MV1 and MV2 generation are presented in Table 2. The irradiation treatment did not affect number of shoots, shoot length and number of leaves because gamma ray mutations occurred on individuals from each treatment randomly. Boxplot (Figure 2 to 7) was used to determine data distribution in each treatment for the analysis of quantitative character so that changes in one individual can be detected on the graph. The existence of different individuals is indicated by the existence of outliers or extreme values on the graph (Amri et al., 2009). The number of shoots in both genotypes has abnormal data distribution in both generation (Figures 2 and 3). White genotype produces fewer shoots than red genotype but, white genotype have individual explants which have the most shoots with P3 treatment dose (LD_{50}). Irradiation treatment in MV2 has extreme values in P1 (LD_{20}) and P3 (LD_{35}) in red genotypes and P1 (LD_{20}) in white genotypes. The existence of extreme values in explant growth following irradiation treatment indicated potential mutants.

Shoot length in MV1 and MV2 generation of both genotypes have asymmetrical data distribution (Figures 4 and 5). The shortest shoots in red genotype were with P2 or LD_{35} (Table 1). The phenotypes of MV2 shoots are likely to be unstable, so changes might still occur in the following generation. Similar to shoot length, number of leaf per explant of both genotypes has an asymmetrical data distribution on

Table 1. Percentage of survived *E. elatior* plantlets after gamma irradiation treatment at five and ten weeks after irradiation

Genotype	Red				White			
Dose (Gy)	0	3 + 3	4 + 4	5 + 5	0	2 + 2	2.8 + 2.8	3.7 + 3.7
Survival (%) at								
5 WAI	100	100	100	100	100	100	100	100
10 WAI	100	92.7	89.3	87.2	100	96	91.8	90.4

Note: WAI = Weeks after irradiation

Table 2. Effect of gamma ray irradiation on number of shoots, shoot length and number of leaf per explant of MV1 and MV2 generation of *E. elatior*[1]

Generation	Genotype	Dose (Gy)	Mean number of shoots ± S.E[2]	Mean shoot length (cm) ± S.E[2]	Mean leaf number ± S.E[2]
MV1	Red	0	1.03 ± 0.12	1.35 ± 0.32	5.33 ± 0.57
		3 + 3	1.00 ± 0.44	1.32 ± 0.13	4.94 ± 0.92
		4 + 4	1.00 ± 0.26	0.95 ± 0.12	4.58 ± 0.45
		5 + 5	1.60 ± 0.46	1.58 ± 0.67	5.67 ± 0.63
	White	0	0.62 ± 0.07	1.17 ± 0.25	4.02 ± 0.74
		2 + 2	0.59 ± 0.26	1.23 ± 0.25	3.29 ± 0.46
		2.8 + 2.8	0.58 ± 0.17	1.36 ± 0.29	4.27 ± 1.46
		3.7 + 3.7	0.61 ± 0.09	1.21 ± 0.18	4.40 ± 0.61
MV2	Red	0	1.07 ± 0.25	1.26 ± 0.15	5.07 ± 0.06
		3 + 3	0.70 ± 0.20	1.20 ± 0.26	4.57 ± 1.03
		4 + 4	0.53 ± 0.32	1.11 ± 0.14	4.46 ± 1.02
		5 + 5	0.80 ± 0.36	1.40 ± 0.17	5.67 ± 0.57
	White	0	0.50 ± 0.26	1.12 ± 0.34	4.07 ± 0.72
		2 + 2	0.30 ± 0.26	1.26 ± 0.23	3.00 ± 0.53
		2.8 + 2.8	0.37 ± 0.06	1.44 ± 0.25	3.80 ± 0.87
		3.7 + 3.7	0.37 ± 0.15	1.14 ± 0.01	3.43 ± 0.75

Note: [1)] Measurements on MV1 and MV2 were conducted at 5 and 10 weeks after irradiation, respectively.

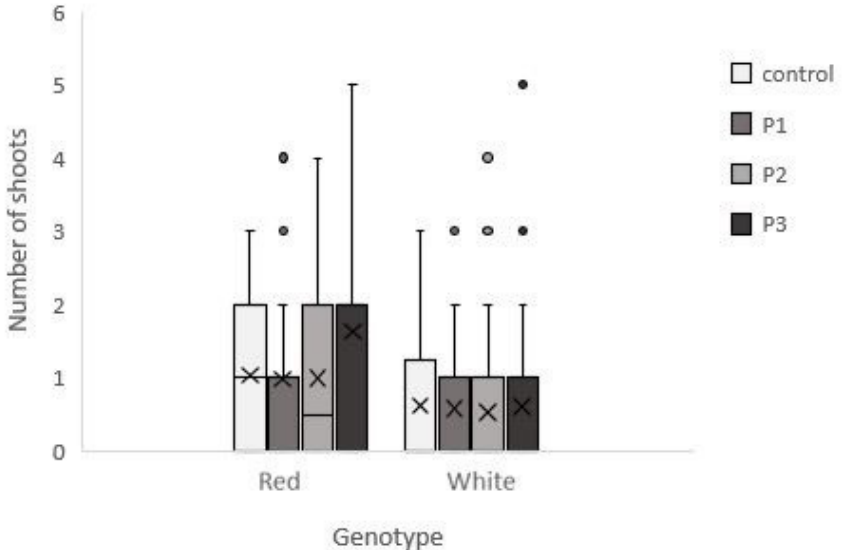

Figure 2. Boxplot number of shoots red and white genotype *E. elatior* MV1.

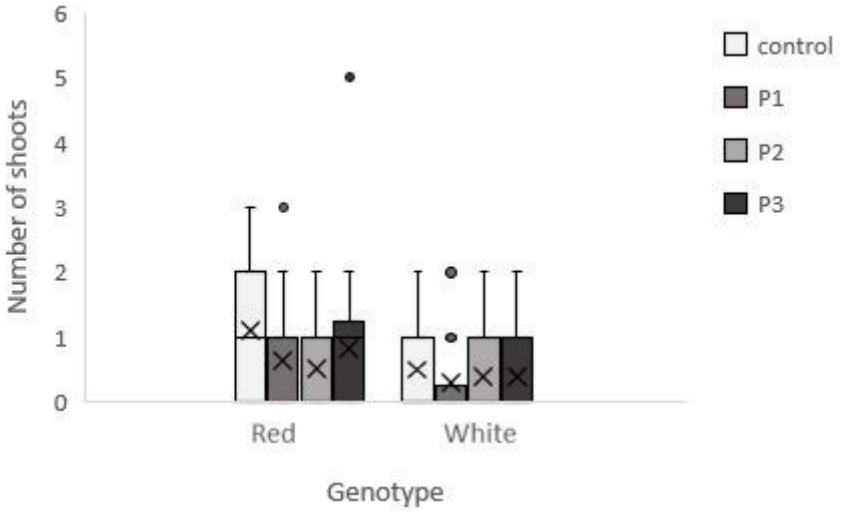

Figure 3. Boxplot number of shoots red and white genotype *E. elatior* MV2.

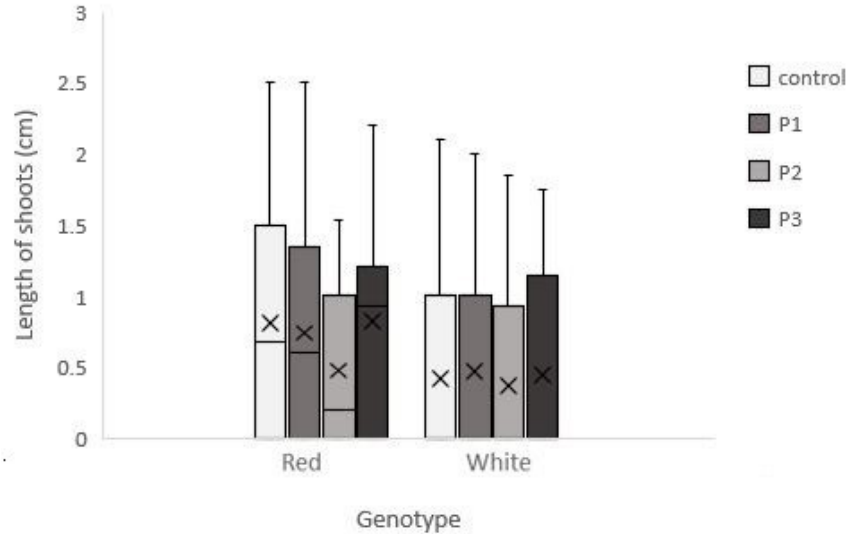

Figure 4. Boxplot length of shoots red and white genotype *E. elatior* MV1

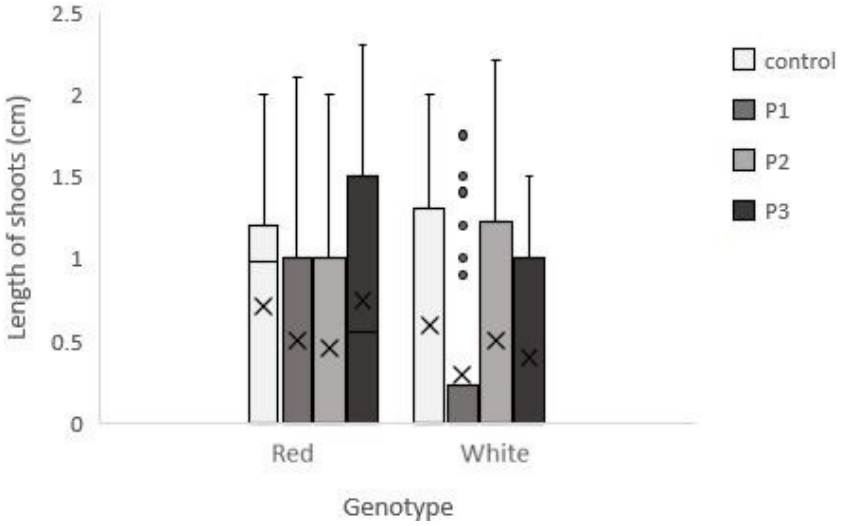

Figure 5. Boxplot length of shoots of red and white genotype *E. elatior* MV2

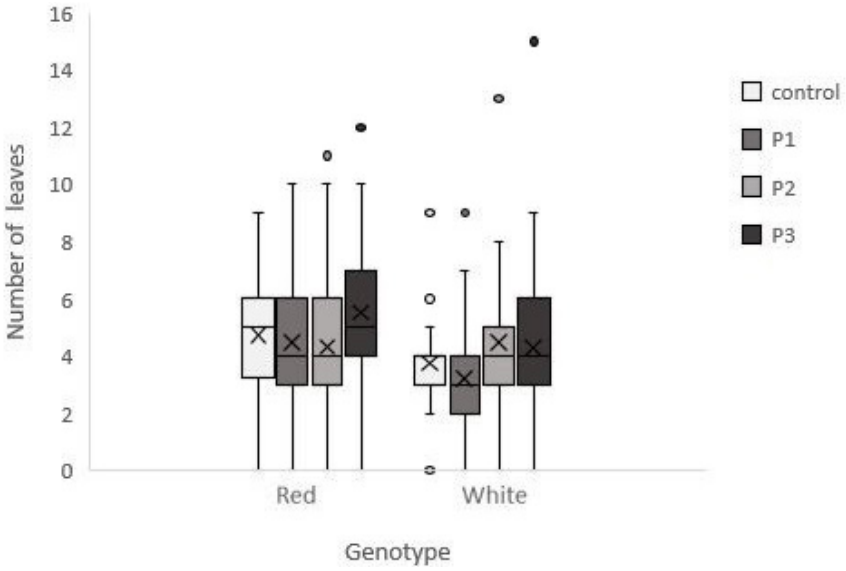

Figure 6. Boxplot number of leaves of red and white genotype *E. elatior* MV1

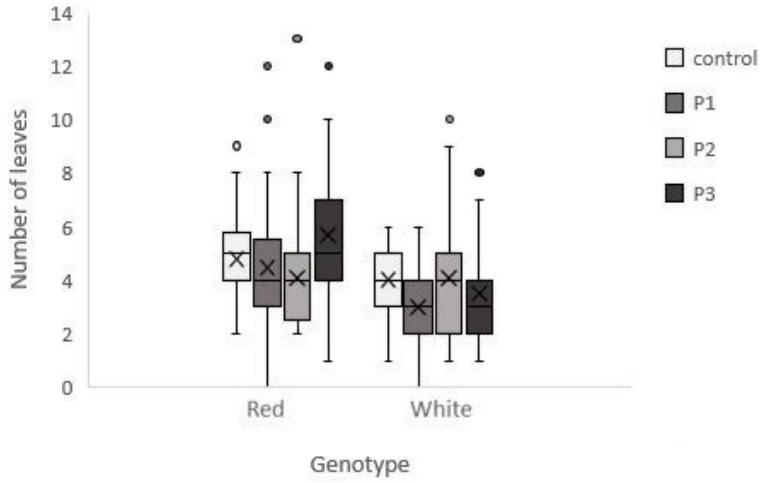

Figure 7. Boxplot number of leaves of red and white genotype *E. elatior* MV2
P1 = LD20 (irradiation at 3+3 Gy for the red genotype; 2+2 Gy for the white genotype); P2 = LD35 (irradiation at 4+4 Gy for the red genotype; 2.8+2.8 Gy for the white genotype); P3= LD50 (irradiation at 5+5 Gy for the red genotype; 3.7+3.7 Gy for the white genotype)

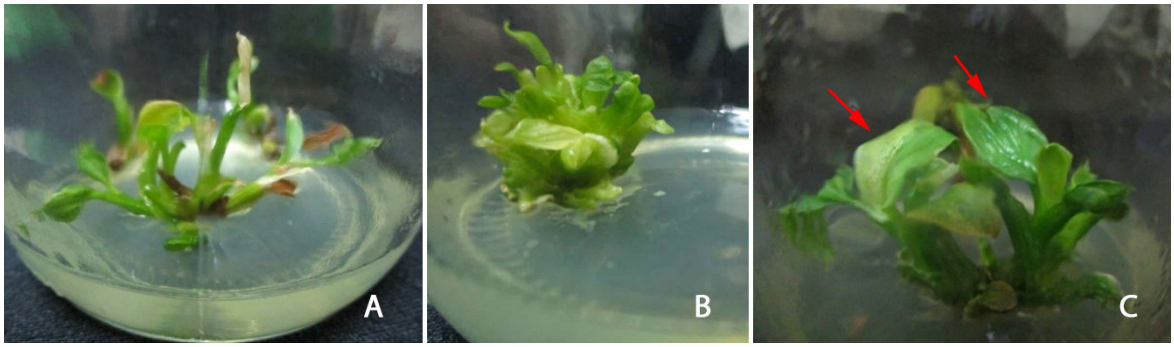

Figure 8. Morphological changes in of *E. elatior* red after gamma irradiation treatment: (A) control; (B) clustered shoots after irradiation at 5 + 5 Gy; (C) variegated and split leaf after irradiation at 5 + 5 Gy.

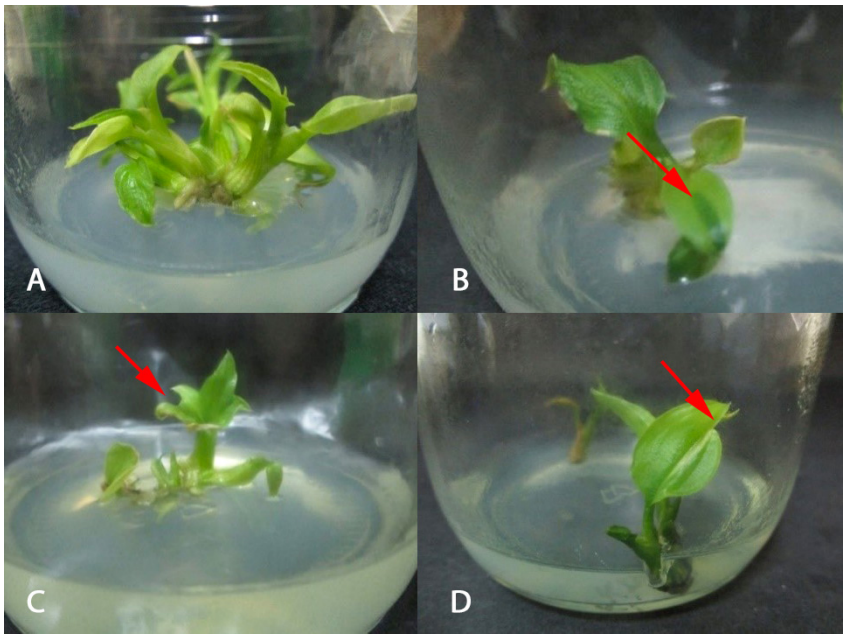

Figure 9. Morphological changes of *E. elatior* white after gamma irradiation treatment: (A) control; (B) a variegated leaf after irradiation at 2.8 + 2.8 Gy; (C) leaf split after irradiation at 3.7 + 3.7 Gy; (D) an asymmetrical leaf after irradiation at 3.7 + 3.7 Gy.

Table 3. Leaf colour classification of MV1 and MV2 *E. elatior* red and white

Red		White	
Dose (Gy)	Colour	Dose (Gy)	Colour
0	Dark green RHS 144A/dark green 141A	0	Dark green RHS 144A/dark green 141A
3 + 3	Dark green RHS 144A/dark green 141A	2 + 2	Dark green RHS 144A/dark green 141A
4 + 4	Dark green RHS 144A/dark green 141A	2.8 + 2.8	Dark green RHS 144A/dark green 141A
5 + 5	Dark green RHS 144A/yellow green RHS 149D	3.7 + 3.7	Dark green RHS 144A/dark green 141A

MV1 and MV2 (Figures 6 and 7). Treatment P1 (LD_{20}) on white genotype had a symmetrical data distribution on MV2. P1 (LD_{20}) and P2 (LD_{35}) treatment have extreme values of 12 and 13 respectively, whereas the white genotype had no extreme values.

Leaf colour and shape was recorded when the plants were 10 WAI (weeks after irradiation). Irradiation treatment produced putative mutant plants in MV1 and MV2 generation. Untreated red genotype (Figure 8 A) had two shoots which can clearly be distinguished from the parent, whereas plantlets irradiated with 5 + 5 Gy produced plantlets with clustered shoots (Figure 8 B) and were difficult to distinguish from their parent, branched or variegated plantlets (Figure 8C). Untreated white genotype has more leaves than

irradiated plantlet (Figure 9 A). Irradiation treatment at 2.8 + 2.8 Gy resulted in variegated leaves (Figure 9 B), whereas treatment 3.7 + 3.7 Gy resulted in branched (Figure 9 C) and asymmetrical leaves (Figure 9 D). The leaf colour from all treatments in both genotypes (Table 3) did not show any differences, except in the treatment dose of 5 + 5 Gy in red genotype which had several plantlets with yellow green leave, or RHS 149D. All changes that occurred in these plantlets have the potential to become mutants.

Determination the radio sensitivity of *in vitro* explants to gamma rays is the initial step of an early screening of variants with possible altered genetic patterns. The critical level of gamma irradiation where mutations were induced is usually within the range of tolerance for regeneration. LD_{50} is recommended irradiation dose which slightly inhibits plant development (Zhou et al., 2006). Based on the results of this study, red genotypes are more sensitive to gamma rays than white genotypes. The percentage of survived plantlets that are irradiated intermittently at LD_{50} (5 + 5 Gy and 3.7 + 3.7) was not show 50% dead plantlets, so as at LD_{35} (4 + 4 Gy and 2.8 + 2.8 Gy) and LD_{20} (3 + 3 Gy and 2 + 2 Gy) (Table 1). It shows that the effects of the LD_{50} acute irradiation was different from LD_{50} intermittent irradiation. Another method to determine the plant's radio sensitivity is based on the value of Reduction Dose (RD_{50}), i.e. a dose that causes 50% of the plant population to experience growth inhibition. The lower the RD_{50} value, the higher the radio sensitivity level (Herison et al., 2008).

The higher dose of irradiation increased damage to the plant, so it can inhibit plant growth including the number and length of shoots. Gamma ray irradiation has high penetration rate so it can reach multicellular layers (Yunus et al., 2013). However, gamma ray can also cause random mutations that can result in physiological damage in cell metabolism, resulting in faster or slower plant growth (Anshori, 2014). Increasing the dose of gamma ray irradiation to a certain level increased the frequency of mutations in a linear manner, but will likely reduce the ability to survive linearly. Putative mutants are generally formed in LD_{50} range (Aisyah et al., 2009).

In this study, gamma ray irradiation treatment at LD_{50} had produced mutants. Additionally, Sianpar et al. (2013) reported that irradiation of *T. flagelliforme* at LD_{50} on 25 Gy produces mutants with taller plants with more shoots than its parents. Anshori et al. (2014) reported that gamma ray irradiation on LD_{50} range in *Curcuma domestica* obtained plants with faster growth than control plants. Dwimahyani and Widiarsih (2010) reported *in vitro* mutagenesis of chrysanthemum (*Dendranthema grandiflora*) at 10 Gy increased the multiplication of shoots by 10% compared to the control plants.

Genetic alteration in first generation after irradiation treatment can easily be seen through morphological changes, so that the plants can be called putative mutants (Anshori et al., 2014). Changes in leaf shape due to gamma ray irradiation are thought to be due to abnormal mutant cells that develop into different tissues or organs compared to those from the parent cells (Cahyo and Dinarti, 2015), while leaf colour changes are caused by disruption of chlorophyll synthesis (Datta, 2012). Gamma irradiation to *Coleus blumei* purple/green resulted in five putative mutants based on changes in leaf colour and pattern (Togatorop et al., 2016), whereas Romeida (2012) reported nine mutants of *S. plicata* orchids. Gamma irradiation at 50 and 70 Gy produced changes in leaf colour and shape of *Curcuma domestica* (Anshori et al., 2014).

Conclusion

Explants of *E. elatior* red irradiated with gamma ray at 5 + 5 Gy and 3.7 + 3.7 Gy had changes in leaf shape; formation of variegated leaves were obtained with gamma ray irradiation at 2.8 + 2.8 Gy and 5 + 5 Gy. Irradiation at 5 + 5 Gy to the red genotype and 3.7 + 3.7 Gy to the white genotype have the potentials to produce putative mutants.

References

Aisyah, S.I, Aswidinnoor H., Saefuddin, A., Marwoto, B., and Sastrosumarjo, S. (2009). Induksi mutasi pada stek pucuk anyelir (Dianthus caryophyllus Linn.) melalui iradiasi sinar gamma. Jurnal Agronomi Indonesia **37**, 62-70.

Amri, A., Junaidi., and Yulmardi. (2009). "Metodologi Penelitian Ekonomi dan Penerapannya" IPB Press.

Anderson, C. (1994). Hawaii tropical botanical garden "a garden in a valley on the ocean". http://www.htbg.com/Zingiberaceae/ETLI-011-3-11-006/ [October 22, 2017].

Anshori, S.R., Aisyah, S.I., and L.K. Darusman. (2014). Induksi mutasi fisik dengan iradiasi sinar gamma pada kunyit (Curcuma domestica Val.). Journal Hortikultura Indonesia **5**, 84-94.

Bala, M., and Kanwar, P.S. (2015). In vitro mutagenesis in rose (Rose hybrida L.) cv. Raktima for novel

traits. Indian Journal of Biotechnology **14**, 525-531.

Cahyo, F.A., and Dinarti, D. (2015). Pengaruh iradiasi sinar gamma terhadap pertumbuhan protocorm like bodies anggrek Dendrobium lasianthera (JJ. Smith) secara *In Vitro*. Jurnal Hortikultura Indonesia **6**, 177-189.

Chan, EWC, Y.Y. Lim, and S.K. Wong. (2011). Phytochemistry and pharmacological properties of Etlingera elatior: A Review. Pharmacognosy Journal **3**, 6-10.

Datta, S.K. (2012). Success story of induced mutagenesis for development of new ornamental varieties. Bioremediation, Biodiversity and Bioavailability **6**, 15-26.

Dwiatmini, K., Kartikaningrum., S., and Sulyo, Y. (2009). Induksi mutasi kecombrang (Etlingera elatior) menggunakan iradiasi sinar gamma. Jurnal Hortikultura **19**, 1-5.

Dwimahyani, I. and Widiarsih, S. (2010). The effects of gamma Irradiation on the growth and propagation of in-vitro chrysanthemum shoot explants (cv. Yellow Puma). Atom Indonesia **36**, 45 – 49.

Herison, C., Rustikawati, Sujono, H.S., and Aisyah, S.I. (2008). Induksi mutasi melalui sinar gamma terhadap benih untuk meningkatkan keragaman populasi dasar jagung (Zea mays L.). Jurnal Akta Agrosia **11**, 57-62.

Mba, C. and Shu, Q.Y. (2011). Gamma irradiation. In "Plant Mutation Breeding and Biotechnology" (Shu, Q.Y., B.P. Foster and H. Nakagawa eds.), pp 92-93. FAO/IAEA.

Murashige T. and Skoog, F.A. (1962). Revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant **15**, 473–497.

Romeida, A. (2012). "Induksi mutasi dengan iradiasi sinar gamma untuk pengembangan klon unggul anggrek Spathoglottis plicata Blume aksesi Bengkulu". Thesis, Departemen Agronomi dan Hortikultura, IPB, Indonesia.

Sabu, M., Aswani, K., and K.P. Smisha. (2013). Reproductive biology of Etlingera elatior (jack.) R. M. Sm. Ornamental torch ginger. International Journal of Plant Animal and Environment Sciences **3**, 75-80.

Sianpar, N.F., Ariandana, W., Rustikawati, and Wilmar, M. (2013). The effects of gamma irradiation on growth response of rodent tuber (Typhonium flagelliforme Lodd.) mutant in in vitro culture. Hayati Journal of Biosciences **2**, 51-56.

Togatorop, E.R. (2016). "Mutasi Induksi pada Coleus spp. dengan Iradiasi Sinar Gamma". Thesis, Department of Agronomy dan Horticulture, IPB, Indonesia.

Van Harten A.M. (2002). Mutation breeding of vegetatively propagated ornamentals. In "Breeding for Ornamentals: Classical and Molecular Approaches" (A. Vainstein, ed.), pp. 105-129. Kluwer Academic Press Boston.

Yunus, M.F., Maheran, A., Mihdzar, A., Azmi, A., S.K. Daud, and A.A. Rashid. (2013). In vitro mutagenesis of Etingera elatior (Jack) and early detection of mutation using RAPD markers. Turkish Journal of Biology **37**, 716-725.