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RESEARCH ARTICLE

Comparative effect of high energy electron beam and ^{137}Cs gamma ray on survival, growth and chlorophyll content in curcuma hybrid 'Laddawan' and determine proper dose for mutations breeding

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ABSTRACT

Curcuma alismatifolia is an ornamental plant with a beautiful flower in the curcuma genus, which is popular in Thailand and other countries. *C. alismatifolia* has been improved as the market needs. This research aimed to induce mutations and to study the effects of electron beam and gamma ray on tissue culture of the *Curcuma* hybrid 'Laddawan.' Eight-weeks-old plantlets were irradiated by gamma ray with a Mark I irradiator at the dose rate of 3.74 Gy/min, at the Nuclear Technology Research Center, Kasetsart University. For the electron beam irradiation, samples were exposed to a high-energy electron beam. The lowest power of this machine is 10 MeV and the lowest radiation dose per cycle is 50 Gy (240 Gy/min). The comparison between the effects of gamma ray and electron beam irradiation at doses of 0, 50, 100 and 200 Gy showed that survival percentage, growth percentage, and chlorophyll content were significantly decreased ($P<0.05$) when radiation dose increased. The survival percentage, growth percentage and chlorophyll content of gamma-irradiated samples were higher than the electron beam-irradiated samples in the same dose. The results indicated the optimum dose range for gamma irradiation of *Curcuma* hybrid 'Laddawan' in tissue culture is 30 – 60 Gy and for electron beams the dose should be less than 50 Gy. New characteristics (variegated leaves and light green leaves) were found in M_1V_2.

Keywords: *Curcuma alismatifolia*; Electron beam; Gamma ray; Mutation; Tissue culture

INTRODUCTION

Curcuma alismatifolia is a tropical flower that can be found in northern and north-eastern Thailand. *C. alismatifolia* is a species of plant in the family Zingiberaceae, genus Curcuma (Department of Agricultural Extension, 2005). A common name is Siam Tulip because they have beautiful leaves and flowers similar to the leaves and flowers of the European tulip. *C. alismatifolia* can be used as cut flowers, potted plants or ornamental plants in beds (National Research Council of Thailand, 2012), which has made them popular in Thailand and other countries. Thailand exports *C. alismatifolia* in the form of undeveloped rhizomes, rhizomes that are growing already, and fresh flowers. Annual export value is 3-6 hundred thousand dollars (Department of Agriculture, 2018). Improvement of *C. alismatifolia* is being worked on continuously to respond to the market demand, by selecting from the genes in nature or through the development of varieties by interspecific hybridization, such as the *Curcuma* hybrid 'Laddawan' of Dynamic Seeds Co., Ltd. It is a hybrid between *C. alismatifolia* and *C. petiolata*. There is a distinctive inflorescence with pink and purple flowers that can be used as cut flowers and potted plants (Fig. 1(A)) (National Research Council of Thailand, 2012). The offspring display traits and characteristics of both parents, but are often sterile, preventing gene flow between the species. Then they cannot interbreed with the parental species. Mutation breeding is a way to induce genetic mutations in plants by using x-rays, gamma-rays, ion beams, laser beams, neutrons and electron beams, which may result in gene mutation and

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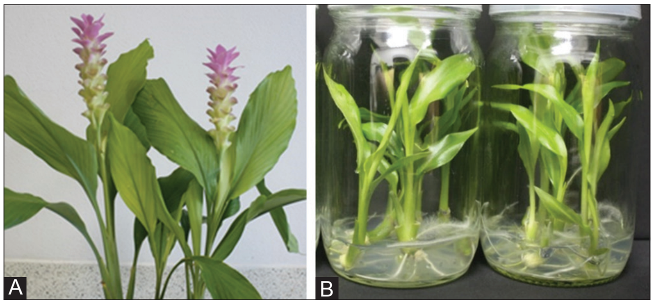

Fig 1. *C.* hybrid 'Laddawan' (A) and pathogen-free plantlets (B).

chromosome aberration, often enabling breeders to gain new varieties. However, mutation is regarded as random and the success of obtaining a desired mutant trait depends on three factors (Hase et al., 2012). Tissue culture allows for large populations for mutagenic treatment, selection, and cloning of selected variants (Ahloowalia, 1995).

In vitro mutagenesis is a good technique for increasing the genetic variation in *C. alismatifolia*. New mutants previously induced by gamma irradiation include 'Chiang Mai Red' and 'Chiang Mai Light Pink' varieties, which were irradiated from 'Chiang Mai Pink' variety (National Research Council of Thailand, 2012). Abdullah et al. (2009) irradiated rhizomes of C. *alismatifolia* var. Pink with 10 different doses, and the (LD_{50}) was approximately 25 Gy. Taheri et al. (2014) irradiated eight different doses of acute gamma irradiation on three varieties of *C. alismatifolia*: Chiang Mai Red, Sweet Pink, Kimono Pink, and one Curcuma hybrid (Doi Tung 554) individual plants were investigated. Until now, no reports are available regarding the use of electron beam for induced mutagenesis in curcuma. The objectives of this study were to determine the optimum dose for induced mutation on tissue culture of the curcuma hybrid 'Laddawan' for mutation breeding using gamma ray and electron beam.

MATERIALS AND METHODS

Plant materials (Curcuma hybrid 'Laddawan')

Pathogen-free plantlets of the *C.* hybrid 'Laddawan' (supported by Chiang Rai Horticultural Crop Research Center, Thailand) were cultured on MS medium (Murashige and Skoog, 1962) with BAP (6-benzylaminopurine) 1 mg/l until enough plantlets (more than 1000) were produced for the experiments.

Gamma and electron beam irradiation

After being sub-cultured to new MS medium (without BAP), eight-week-old plantlets of *C.* hybrid 'Laddawan' were exposed to radiation treatments. The radiation doses were 0, 50, 100, 150 and 200 Gy, with 3 replications for each treatment, 20 plantlets per replication. For gamma irradiation, the plantlets were exposed to gamma radiation using a Mark I Gamma Irradiator with a ^{137}Cs source at the Nuclear Technology Research Center, Kasetsart University, Bangkok, Thailand, at a dose rate of 3.74 Gy/min. For electron beam irradiation, the samples were exposed to electron beams with a high-energy electron accelerator at the National Institute of Nuclear Technology (Public Organization), Ministry of Science and Technology. The lowest power of this machine is 10 MeV and the lowest radiation dose per cycle is 50 Gy (at a dose rate of 240 Gy/min). After irradiation, the plantlets were sub-cultured to new MS medium for the M_1V_1 generation. At 60 and 90 d after irradiation the number of surviving plantlets were recorded to calculate the median lethal dose (LD_{50}, (50% lethal dose at 60 and 90 d after irradiation)), the number of new shoots were recorded to calculate the median decreasing growth rate (GR_{50}) and the characteristics of abnormalities after radiation in the M_1V_1 generation were observed. At 90 d after irradiation, new shoots (M_1V_2) were subcultured to new media. Desirable variations were recorded and selected.

Chlorophyll content measurement of C. hybrid 'Laddawan' leaves in the M_1V_1 generation

Chlorophyll content was determined in leaves of M_1V_1 plantlets at 90 d after gamma and electron beam irradiation based on an adapted method described by Hipkins and Baker (1986). The chlorophyll content was measured in triplicate for each treatment.

Experimental design and statistical analysis

The experimental design was a factorial in a completely randomized design (Factorial in CRD) with two factors: radiation type (gamma ray and electron beam) and radiation dose. The radiation doses were 0, 50, 100, 150 and 200 Gy, with 3 replications for each treatment, 20 plantlets per replication. The data were analyzed using analysis of variance, after which means were compared using the least significant difference (LSD). The analyses were facilitated by the R program (Jompuk, 2012).

RESULTS AND DISCUSSION

Effects of gamma and electron beam irradiation on the survival of *C.* hybrid 'Laddawan'

At 60 d after irradiation, the results showed that gamma and electron beam exposure affected the survival of *C.* hybrid 'Laddawan' plantlets. The irradiated samples had a percentage of survival less than the control, but it was not possible to calculate the LD_{50} at 60 d of the two types of radiation (Fig. 3). The samples irradiated with gamma rays had a higher survival percentage than those exposed to electron beam at the same dose. The

Table 1: Survival percentage of *C.* hybrid 'Laddawan' *in vitro* plantlets at 60 and 90 d after gamma and electron beam irradiation compared with the control

Dose (Gy)	Survival percentage at 60 days after irradiation (as % of control)			Survival percentage at 90 days after irradiation (as % of control)		
	gamma	e- beam	average	gamma	e- beam	average
0	100 a	100 a	100 a	100	100	100 a[1/]
50	86.11 b	66.67 e	76.39 b	63.89	51.85	57.87 b
100	81.48 bc	72.22 cde	76.85 b	40.74	36.11	38.43 c
150	76.19 cd	56.67 f	66.43 c	28.57	18.89	23.73 d
200	69.64 de	52.38 f	61.01 c	22.02	14.29	18.15 e
average	82.69 a	69.59 b		51.04 a	44.23 b	
F-test dose	**			**		
F-test rad	**			**		
F-test dose x rad	**			ns		
C.V. (%)	7.17			9.06		

**Significant at 1% level.
ns Non-significant difference
1/ Different letters in the same column mean that the data are statistically different from the least significant difference ($p < 0.05$).

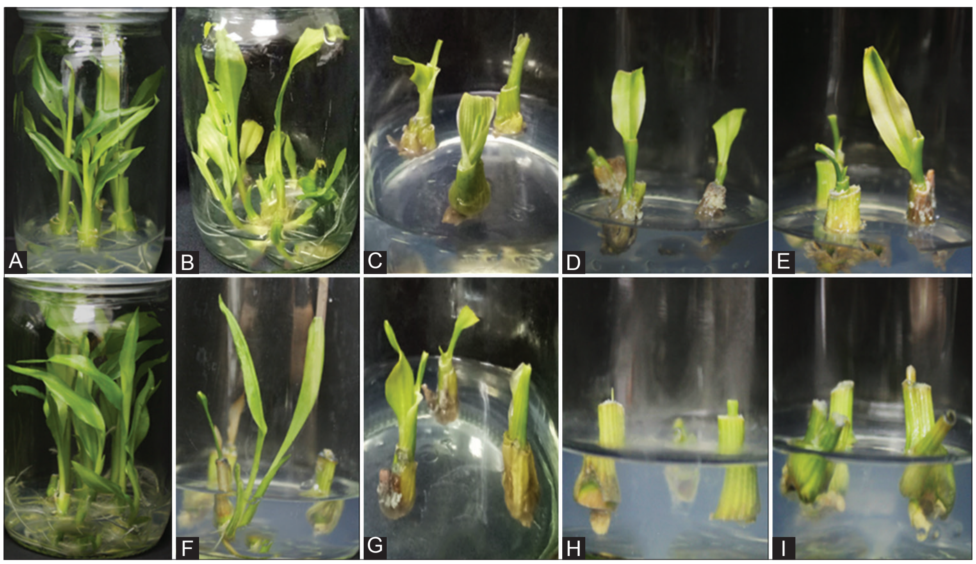

Fig 2. Control and irradiated samples at 90 d after irradiation: (A) Control; gamma irradiation, (B) 50 Gy; (C) 100 Gy; (D) 150 Gy; (E) 200 Gy and electron beam irradiation (F) 50 Gy; (G) 100 Gy; (H) 150 Gy; (I) 200 Gy.

interaction between the radiation dose and the type of radiation was a highly significant difference (Table 1). At 90 d after irradiation (Fig. 2), the results showed that the doses of gamma and electron beam affected the survival percentage with highly significant differences ($p < 0.01$). The LD_{50} at 90 d of gamma and electron beam irradiation were 80 and 55 Gy, respectively (Fig. 3). The results of the current study differed from Lamseejan et al. (2001). Taheri et al. (2014) studied the effects of different doses of acute gamma irradiation on three varieties of *Curcuma alismatifolia*. For electron beam irradiation it was found that the survival percentage decreased when the radiation dose increased, in accordance with the results of experiments on electron beam irradiation in rice (Guo et al., 1982), barley (Xu et al.,1983), soybean (Li et al., 1988), sorghum (Lu et al., 1995) and azuki bean (Luo et al., 2012). The LD_{50} at 90 d of electron beam was lower than the gamma-ray. This

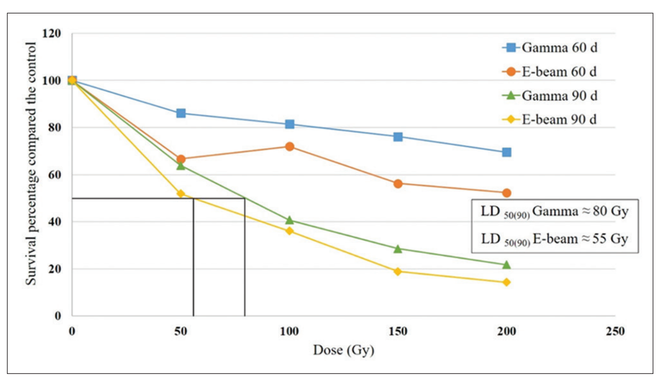

Fig 3. The relationship between the dose of gamma ray, electron beam and survival percentage of C. hybrid 'Laddawan' in vitro plantlets at 60 and 90 d after irradiation compared with the control.

was in agreement with Veni et al. (2017), who studied the effects of electron beam and gamma ray in blackgram and

Table 2: Growth rate percentage of *C.* hybrid 'Laddawan' *in vitro* plantlets at 60 and 90 d after acute gamma and electron beam irradiation compared with the control

Dose (Gy)	Growth rate percentage at 60 days after irradiation (as % of control)			Growth rate percentage at 90 days after irradiation (as % of control0		
	gamma	e - beam	average	gamma	e - beam	average
0	100	100	100 a	100 a	100 a	100 a[1/]
50	55.56	57.41	56.48 b	60.38 b	29.1 c	44.74 b
100	0.00	0.00	0 c	0 d	0 d	0 c
150	0.00	0.00	0 c	0 d	0 d	0 c
200	0.00	0.00	0 c	0 d	0 d	0 c
average	31.11	31.48		32.08 a	25.82 b	
F-test dose	**			**		
F-test rad	ns			**		
F-test dose x rad	ns			**		
C.V. (%)	12.32			9.65		

**Significant at 1% level.
ns Non-significant difference
[1/] Different letters in the same column mean that the data are statistically different from the least significant difference test.

found that the LD_{50} dose for electron beam was around 300 Gy whereas, the LD_{50} dose for gamma rays was around 320 Gy. Linear energy transfer (LET) is defined as the energy deposited to the target material per unit distance when ionizing radiation passes through it. Different kinds of radiation have different energy transfer patterns. The mutation effects of radiation are known to be a function of their LET. Both gamma rays and electron beams have low LETs of around 0.2KeV/µm (Magori et al. 2010). However, electron beam has a higher dose rate compared to gamma rays and is administered as short pulses while gamma irradiation is continuous (Hoglund et al. 2000).

Effects of gamma and electron beam irradiation on the growth rate of *C.* hybrid 'Laddawan'

The effect of radiation on growth was assessed by counting the number of new plantlets at 60 and 90 d after irradiation and calculating the growth rate (Table 2, Fig. 4). Previous findings supports our study (Jompuk et al., 2009; Limtiyayotin et al., 2018; Lamseejan et al., 2002). The relative growth percentage with respect to non-irradiated control in the M_1V_1 generation was found to be reduced in a dose dependent manner (Table 2). However, there was no difference in the relative growth percentage of plantlets irradiated with either electron beam or gamma irradiation with similar doses. Waje et al. (2009) showed that broccoli seeds irradiated with the same dose of either electron beam or gamma rays had similar germination percentages. They also reported a decrease in sprout length of germinated seedlings with increase in dose of either gamma ray or electron beam. Our findings were similar to these results.

The GR_{50} at 90 d of gamma and electron beam irradiation were 59 and 36 Gy, respectively (Fig. 4). The results showed that, for gamma irradiation, the growth rate increased when the time after irradiation increased, but for electron beam irradiation, the growth rate decreased when the time after irradiated increased, because of electron beam has a higher dose rate when compared to gamma rays. The higher the dose-rate, the greater is the relative biological effectiveness (RBE). The absorbed dose rate of electron beam on biomaterials may reach 10^{10} $Gy.s^{-1}$, which is much higher than gamma rays (usually under 60 $Gy.s^{-1}$) (Zhu et al., 2008). For irradiation effects on cell division, the cells will be temporarily stopped from dividing and after some time they will be able to divide as before. It depends on the type of radiation, the dose and dose rate of irradiation (Yamaguchi, et al., 2010). In this study, the plantlets irradiated with both radiation types at doses of 100-200 Gy died (Table 2). It may be that the high radiation doses inhibit plant growth due to the meristem being destroyed or inhibiting the cell division (Zhu et al., 2008, Joshi-Saha et al., 2015 and Yadav, 2016)

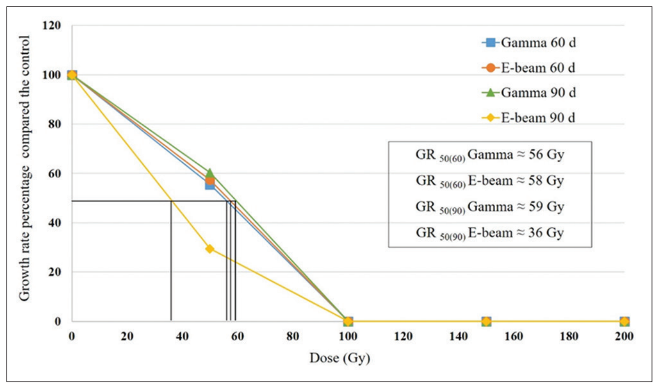

Fig 4. The relationship between the dose of gamma ray, electron beam and growth rate percentage of C. hybrid 'Laddawan' at 60 and 90 d after irradiation compared with the control.

Effects of gamma and electron beam irradiation on chlorophyll content in leaves of C. hybrid 'Laddawan' M_1V_1 plantlets

The chlorophyll contents were measured for all samples exposed to any doses except the samples that were exposed to electron beam at doses of 150 and 200 Gy. Statistical

Table 3: Chlorophyll content of leaves of *C.* hybrid 'Laddawan' M_1V_1 plantlets

Dose (Gy)	Chlorophyll a (mg/g of fresh leaf weight)			Chlorophyll b (mg/g of fresh leaf weight)			Total chlorophyll (mg/g of fresh leaf weight)		
	gamma	electron beam	average	gamma	electron beam	average	gamma	electron beam	average
0	1.162 a	1.162 a	1.162 a	0.513	0.513	0.513 a	1.690 a	1.690 a	1.690 a
50	0.737 b	0.447c	0.592b	0.327	0.227	0.277 b	1.074 b	0.681c	0.877 b
100	0.307 cd	0.118 e	0.213 c	0.149	0.054	0.1020 c	0.461 d	0.174e	0.318 c
150	0.202 de	NA	0.202 cd	0.091	NA	0.0910 c	0.297 de	NA	0.297 cd
200	0.084 e	NA	0.084 d	0.037	NA	0.0370 c	0.122 e	NA	0.122 d
average	0.498	0.576		0.223	0.265		0.7290	0.848	
F-test dose	**			**			**		
F-test rad	ns			ns			ns		
F-test dose x rad	*			ns			*		
C.V. (%)	15.32			18.72			16.13		

*Significant at 5% level.
**Significant at 1% level.
ns Non-significant difference
1/ Different letters in the same column mean that the data are statistically different from the least significant difference test.

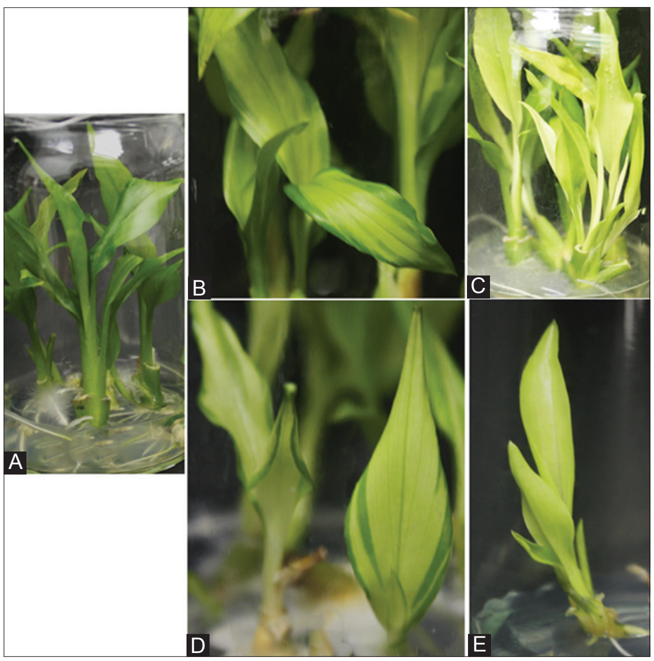

Fig 5. Morphological variation in the M_1V_2 generation of C. hybrid 'Laddawan': gamma irradiation (variegated leaves (B) and light green leaves (C)) and electron beam irradiation (variegated leaves (D) and light green leaves (E)) compared to control (A).

analysis showed highly significant decreases in chlorophyll a, chlorophyll b and total chlorophyll content in the leaves of plants derived from irradiated plantlets. The irradiated samples (both gamma and electron beam) had chlorophyll content that was less than control and decreased when radiation dose increased. Comparing between gamma rays and electron beam, there were no significant differences in chlorophyll a, chlorophyll b and total chlorophyll content. As for the interaction between the dose of radiation and the type of radiation, the chlorophyll a and the total chlorophyll content were significantly different ($p < 0.05$) but the chlorophyll b content was not significantly different (Table 3). Earlier reports supports our findings (Dale et al., 1997; Kalimullah et al., 2003); Ling et al., 2008; Bae et al., 2001; Saha et al., 2010).

Variations in the M_1V_2 generation

Morphological variations in the M_1V_2 generation were observed, but only the 50 Gy treatment samples of gamma and electron beam produced new plantlets (M_1V_2). There were some plants with variegated leaves and light green leaves for both radiation types (Fig. 5). The results of this study were consistent with those of Priya et al. (2014), who reported that in gamma irradiation on tissue culture of turmeric, the chlorophyll mutations were viridis and xantha. In Abdullah et al. (2009)'s study on gamma irradiation on rhizomes of *C. alismatifolia*, the results showed that the variations observed were two inflorescences in the same stem, two real flowers in the same bract, changing the color intensity of flowers and chlorophyll mutation on leaves. For electron beam, in a study by Luo et al., (2012) dry seeds of azuki bean (*Vigna angularisi*) were irradiated by electron beam at 100, 300, 600, 700 and 900 Gy, respectively.

CONCLUSIONS

The tested doses of gamma rays and electron beam affected the survival and growth rate of *C.* hybrid 'Laddawan.' The survival percentage and growth rate decreased when the dose of radiation increased. The gamma irradiated samples had a higher survival percentage and growth rate than the electron beam irradiated samples. The LD_{50} at 90 d of gamma and electron beam irradiation were 80 and 55 Gy, respectively. The $GR_{50(90)}$ of gamma and electron beam irradiation were 58 and 36 Gy, respectively.

From this research the appropriate dose of gamma radiation to be used for inducing mutation in tissue cultures of *C.* hybrid 'Laddawan' ranged from 30 – 60 Gy, and for electron beams should be less than 50 Gy.

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Author's contributions

This work is part of the M.Sc. thesis of Chutiphorn Tosri (CT) advised by Peeranuch Jompuk (PJ). CT and PJ have designed the study, conducted the experimental work, and analyzed data. PJ wrote and revised the manuscript. Katarut Chusreeaeom is the committee of the M. Sc. thesis. Mayuree Limtiyayotinand Natnichaphu Sukin are the scientists who take care the instruments and tissue culture lab.

REFERENCES

Abdullah, T. L., J. Endan and B. M. Nazir. 2009. Changes in flower development, chlorophyll mutation and alteration in plant morphology of *Curcuma alismatifolia* by gamma irradiation. Am. J. Appl. Sci. 6(7): 1436-1439.

Ahloowalia, B. 1995. *In vitro* Mutagenesis for the Improvement of Vegetative Propagated Plants. Induced Mutations and Molecular Techniques for Crop Improvement. Proceeding of International Symposium. IAEA and Food Agriculture Organization of the United Nations, IAEA SM-340, Vienna, pp.531-541.

Bae, C. H., T. Abe, T. Matsuyama, N. Fukunishi, N. Nagata, T. Nakano, Y. Kaneko, K. Miyoshi, H. Matsushima and S. Yoshida. 2001. Regulation of chloroplast gene expression is affected in ali, a novel tobacco albino mutant. Ann. Bot. 88: 545-553.

Dale, M. F., D. W. Griffiths, H. Bain and B. A. Goodman. 1997. The effect of gamma irradiation on glycoalkaloid and chlorophyll synthesis in seven potato cultivars. J. Sci. Food Agric. 75: 141-147.

Department of Agricultural Extension. 2005. Pathumma. The Agricultural Co-operative Federation of Thailand. LTD. Bangkok, Thailand.

Department of Agriculture. 2018. Strategy for the development of Pathumma Research, 2016-2018. Available from: http://www.doa.go.th/hortold/images/ stories/strategyplanthort/strategypratumma.doc. [Last accessed on 2018 Aug 20].

Guo, B. J., Y. Y. Wu and J. H Ruan. 1982. Studies on the mutagenic effect of 5 MeV electron irradiation on rice. Acta Genet. Sin. 9: 461-467.

Hase, Y., Y. Akita, S. Kitamura, I. Narumi and A. Tanaka. 2012. Development of an efficient mutagenic technique using ion beam: Towards more controlled mutation breeding. Plant Biotech. 29: 193-200.

Hipkins, M. F. and N. R. Baker. 1986. Photosynthetic Energy Transduction: A Practical Approach. IRL Press, Arlington.

Hoglund, E., E. Blomquist, J. Carlsson and B. Stenerlow. 2000. DNA damage induced by radiation of different linear energy transfer: initial fragmentation. Int. J. Radiat. Biol. 76: 539-547.

Joshi-Saha, A., K. S. Reddy, V. C. Petwal and J. Dwivedi. 2015. Identification of novel mutants through electron beam and gamma irradiation in chickpea (*Cicer arietinum* L.). J. Food Legumes 28: 1-6.

Jompuk, C. 2012. Statistics: Experimental Planning and Data Analysis in Plant Research with R Stat. Kasetsart University Press. Bangkok, Thailand.

Jompuk, P., C. Jompuk, A. Bunjongpetch and P. Tangpong. 2009. Effects of acute and chronic gamma irradiation on tissue culture of Cryptocoryne wendtii "brown". Kasetsart J. Nat. Sci. 43: 254-260.

Kalimullah, M., J. U. Gaikwad, S. Thomas, A. Sarma and P. B. Vidyasagar. 2003. Assessment of 1H heavy ion irradiation induced effects in the development of rice (*Oryza sativa* L.) seedlings. Plant Sci. J. 165: 447-454.

Lamseejan, S., P. Jompuk, A. Wongpiyasatid, P. Kwanthammachart and R. Meesat. 2001. Improvement of Ornamental Plants through Induced Mutations. In: FAO/IAEA Seminar on Mutation Techniques and Molecular Genetics for Tropical and Subtropical Plant Improvement in Asia and the Pacific Region. Makati City, Philippines, pp. 19-20.

Lamseejan, S., P. Jompuk and S. Deeseepan. 2002. Mutation Induction in Chrysanthemum through *in vitro* Acute and Chronic Irradiations with Gamma Rays: Proceedings of the FNCA Workshop on Plant Mutation Breeding 2001-Molecular-Biological Techniques. Bangkok, Thailand, pp. 149-156.

Limtiyayotin, M., C. Tosri, N. Sukin and P. Jompuk. 2018. Effects of acute gamma irradiation on *in vitro* culture of *Exacum affine* Balf.f. ex Regel. Agric. Nat. Resour. 52(2): 121-124.

Li, G. Q., S. H. Yu and M. Li. 1988. Study of the effect of electron beam on soybean radiation mutation. Acta Agric. Univ. Jilinensis 10: 57-62.

Ling, A. P. K., J. Y. Chia, S. Hussein and A. R. Harun. 2008. Physiological responses of Citrus sinesis to gamma irradiation. World Appl. Sci. J. 5: 12-19.

Lu, W., Y. M. Su and G. Y. Song. 1995. Comparison of electron beam and γ ray irradiated seeds of Sorghum. J. Nucl. Agric. Sci. 16: 160-163.

Luo, W. X., Y. S. Li, B. M. Wu, Y. E. Tian, B. Zhao, L. Zhang, K. Yang and P. Wan. 2012. Effects of electron beam radiation on trait mutation in azuki bean (*Vigna angularis*). Afr. J. Biotechnol. 11: 12939-12950.

Magori, S., A. Tanaka and M. Kawaguchi. 2010. Physically induced mutation: Ion beam mutagenesis. In: Kahl, G and K. Meksem., (Eds.), The Handbook of Plant Mutation Screening. WILEY VCH Verlag GmbH and Co. KGaA, Weinheim, pp 3-16.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plant 15: 473-497.

National Research Council of Thailand. 2012. Pathumma: Breeding and Application. Amarin Printing and Publishing Public Company Limited. Bangkok, Thailand.

Priya, I. N., V. Devappa, M. S. Kulkarni and G. Prabhulinga. 2014. Effect of gamma radiation on growth of turmeric (*Curcuma longa* L.) cv. Salem explants. Karnataka J. Agric. Sci. 27(2): 152-155.

Saha, P., Raychaudhuri, S. S., Chakraborty, A., Sudarshan, M. 2010. PIXE analysis of trace elements in relation to chlorophyll concentration in Plantago ovata Forsk. Appl. Radiat. Isot. 68: 444-449.

Taheri, S., T. L. Abdullah, Z. Ahmad and N. A. P. Abdullah. 2014. Effect of acute gamma irradiation on *Curcuma alismatifolia* varieties and detection of DNA polymorphism through SSR marker. Biomed Res. Int. 2014: 1-18.

Veni, K., C. Vanniarajan, and J. Souframanien. 2017. Probit analysis and effect of electron beam and gamma rays in blackgram (*Vigna mungo* (L.) Hepper). Electron. J. Plant Breed. 8: 950-955.

Waje, C. K., S. Y. Jun, Y. K. Lee, K. D. Moon, Y. H., Choi and J. H. Kwon. 2009. Seed viability and functional properties of broccoli sprouts during germination and postharvest storage as affected by irradiation of seeds. J. Food Sci. 74: C370-4.

Xu, X. C., X. H. Zhou, G. Z. Li, G. Q. Dai, P. D. Wang and B. L. Wang. 1983. Inducing mutation by electron beam in barley. Appl. Energy Agric. 4: 44-50.

Yadav, V. 2016. Effect of gamma radiation on various growth parameters and biomass of *Canscora decurrens* Dalz. Int. J. Herb. Med. 4(5): 109-115.

Yamaguchi, H., A. Shimizu, Y. Hase, A. Tanaka, N. Shikazono, K. Degi and T. Morishita. 2010. Effects of ion beam irradiation on mutation induction and nuclear DNA content in chrysanthemum. Breed. Sci. 60: 398-404.

Zhu, H., J. Xu, S. Li, X. Sun, S. Yao and S. Wang. 2008. Effect of high-energy-pulse-electron beam radiation on biomolecules. Sci. China Ser. B. 51: 86-91.