Kasetsart J. (Nat. Sci.) 43 : 449 - 457 (2009)

Effects of Acute and Chronic Gamma Irradiations on *In vitro* Culture of *Anubias congensis* N.E. Brown

Pakorn Tangpong[1], Thanya Taychasinpitak[2], Choosak Jompuk[3] and Peeranuch Jompuk[1,4]*

ABSTRACT

Plantlets of *Anubias congensis* N.E. Brown cultured on MS medium were irradiated with gamma rays for both acute and chronic treatments of 0, 20, 40, 60, 80 and 100 Gy at a dose rate of 264 Gy/hr (acute) and 0, 14.34, 28.60, 31.24, 42.90, 51.16, 65.55, 82.42, 91.69, 105.99 and 120.30 Gy at a dose rate of 0.71 Gy/hr (chronic). The calculated $LD_{50(60)}$ for the acute irradiation was 32.12 Gy. It was not possible to calculate the $LD_{50(60)}$ for the chronic irradiation because the survival rate was 100% for all treatments. Following acute gamma irradiation, the number of roots, number of shoots, number of leaves, length and width of leaves and length of roots in the M_1V_1 generation of *A. congensis* N.E. Brown plantlets were less than the control. With chronic gamma irradiation, there was no difference in most parameters with the exception of the 105.99 and 120.30 Gy treatments, in which the number of roots was greater than the control. Some abnormalities observed in the M_1V_1 generation exposed to acute irradiation that were genetically transferred to the M_1V_3 generation included narrow leaves, undulate leaf margins, and dwarfism with light green leaves. The mutation frequencies for these characters were 11.67, 26.67 and 6.67% for a dose of 20, 40 and 60 Gy, respectively. For the samples exposed to chronic irradiation, mutations observed in the M_1V_3 generation were dwarfism and albinism. The mutation frequencies were 1.67, 1.67, 3.33, 5.00, 11.67 and 10.00% at a treatment dose of 14.34, 28.60, 31.24, 91.69, 105.99 and 120.30 Gy, respectively. No mutation could be detected in the M_1V_3 generation irradiation with chronic gamma rays at 42.90, 51.16, 65.55 and 82.42 Gy. The potential mutants of *A. congensis* N.E. Brown in this research were the undulate leaf margins and dwarfism with light green leaves. All the mutation characters were maintained through tissue culture.

Key words: *Anubias congensis* N.E. Brown, gamma rays, acute irradiation, chronic irradiation, tissue culture

INTRODUCTION

Aquatic plants in the genus *Anubias* are popular among aquatic plant enthusiasts who enjoy collecting novelties. They are widely used for decorating aquariums and making ornamental fish healthier in more natural surroundings. *Anubias spp*. are monocotyledonous angiosperms in the

1 Department of Applied Radiation and Isotopes, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand.
2 Department of Horticulture, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand.
3 Department of Agronomy, Faculty of Agriculture, Kasetsart University, Kamphaeng Saen, Nakhon Pathom, 73140, Thailand.
4 Gamma Irradiation Service and Nuclear Technology Research Center, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand.
* Corresponding author, e-mail: fsciprk@ku.ac.th

Received date : 20/02/09 Accepted date : 23/04/09

family Araceae. The stem is an underwater rhizome. The leaves are ovate to lanceolate, with a dark green color. Both perfect flowers and imperfect flowers are formed. The flowers are small and apetalous. *Anubias spp*. grow well at temperatures of 20-25°C at pH 6.5-7.4 ((M¸hlberg, 1982) and the genus is well adapted to growing in the climatic conditions of Thailand. The price of *Anubias* plants is rather high and they grow slowly (Rataj and Horeman, 1977). Several different propagation methods may be used, including dividing and tissue culture. Using radiation-induced mutations, especially from ionizing radiations such as gamma rays, X-rays or neutrons, is another method to increase morphological and genetic diversities in aquatic plant species. Gamma radiation has been widely used to induce mutations in many kinds of plants, for example, *Antirrhinum majus* (Cuany *et al*., 1985) *Chrysanthemum morifolium* (Jompuk *et al*., 2001) and *Saintpaulia ionantha* (Seneviratne and Wijesundara, 2007; Wongpiyasatid *et al*., 2007). Gamma rays transfer energy to molecules in the cells of plants, especially the DNA molecules that contain the genetic information, resulting in point mutations (Lamseejan *et al*., 2000). However, if the damage to the DNA is so severe that it cannot be repaired by the cell, then the cell will die (Masami, 2002). Pongchawee *et al*. (2007) studied the effects of acute gamma radiation on *Anubias nana* and found that different doses of radiation caused changes in the leaves and size of the plant, such as variegated leaves and dwarf plants. Wang *et al*. (2006) studied the effects of chronic gamma irradiation on tissue-cultured sweet potato (*Ipomoea batatas* (L) Lam.) and reported mutations involving leaf vein color and leaf shape. The objective of this research was to study the effect of both acute and chronic gamma irradiation on the survival, growth rate and mutation of *A. congensis* N.E. Brown plantlets grown in tissue culture.

MATERIALS AND METHODS

Propagation of *Anubias congensis* N.E. Brown in tissue culture

Pathogen-free plantlets of *A. congensis* N.E. Brown were cultured on MS medium (Murashige and Skoog, 1962) supplemented with BA (N^6-benzyladenine) 8.88 µmol l^{-1} until enough plantlets were produced for the experiments. Plantlets were transferred to MS hormone-free medium before being subjected to radiation treatments.

Acute and chronic gamma irradiations

For the acute gamma irradiation treatments, plantlets of *A. congensis* N.E. Brown raised *in vitro* were exposed to gamma radiation at 0 (control), 20, 40, 60, 80 and 100 Gy (a dose rate of 264Gy/hr from a Cs-137 source using a MARK I Research Irradiator). *In vitro* plantlets of *A. congensis* N.E. Brown were exposed to chronic gamma rays at a dose rate of 0.71 Gy/hr, placed at a distance of 2 m from a Co-60 source in the Gamma Room at the Gamma Irradiation Service and Nuclear Technology Research Center, Faculty of Science, Kasetsart University. The samples received 0 (control), 14.34, 28.60, 31.24, 42.90, 51.16, 65.55, 82.42, 91.69, 105.99 and 120.30 Gy of radiation for the different treatment levels.

Statistical analysis

The experiment followed a completely randomized design (CRD) with three replications for each treatment and 20 samples for each replication. Plantlet survival was recorded to calculate the $LD_{50(60)}$ (50% lethal dose at 60 days after irradiation). The number of leaves, number of shoots, leaf width and length and root length were recorded at 60 days after irradiation for the M_1V_1 generation and types of mutations were recorded for the M_1V_3 generation. The data were analyzed using analysis of variance (ANOVA), after which means were tested by Duncan's new multiple range test (DMRT) and compared.

RESULTS AND DISCUSSION

Effects of acute and chronic gamma irradiations on the survival of *A. congensis* N.E. Brown plantlets

When the plantlets of *A. congensis* N.E. Brown were exposed to acute gamma radiation at the rates of 0, 20, 40, 60, 80 and 100 Gy, it was found that 60 days after the treatment, the percentage of survival plants was negatively correlated with the amount of radiation received. None of the plantlets exposed to 80 Gy or more survived (Table 1). The $LD_{50(60)}$ was 32.12 Gy. This differed from the findings of Pongchawee *et al*. (2007), who were unable to calculate the $LD_{50(30)}$ for *Anubias* spp. on exposure to gamma radiation because none of the samples died after 30 days following exposure to 0-120 Gy of gamma radiation. However, in this experiment the survival rate was recorded after 60 days instead of 30 days for calculating the 50% lethal dose.

For the chronic radiation experiment, however, it was not possible to calculate the $LD_{50(60)}$ because none of the samples died. This may have been because the radiation rate for chronic irradiation was rather low at 0.71 Gy/hr, compared to 264Gy/hr for the acute irradiation experiment. At the lower dose rate of radiation the plant cells were less likely to be fatally damaged; they were able to repair themselves and return to the normal cell cycle (Klein and Klein, 1971).

Table 1 Effects of acute gamma irradiation on *A. congensis* N.E. Brown at 60 days after irradiation.

Dose (Gy)	Total no. of treated plantlets	No. of survival plantlets	Plantlet survival (% of control)
0	60	60	100
20	60	53	88.30
40	60	16	16.67
60	60	4	6.67
80	60	0	0
100	60	0	0

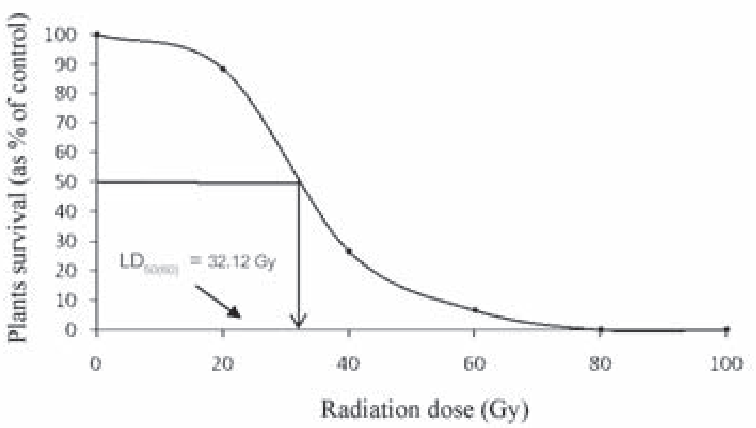

Figure 1 Relationship between radiation dose and survival of *A. gongensis* N.E. Brown plantlet 60 days after gamma irradiation.

Effects of chronic and acute gamma irradiations on the growth of *A. congensis* N.E. Brown

When the plantlets of *A. congensis* N.E. Brown were exposed to acute gamma irradiation, the number of new shoots, roots and leaves decreased as the radiation doses increased to a statistically significant difference. Plantlets exposed to 40 Gy or more did not develop any new roots. Plantlets exposed to 80 Gy or more did not develop any new shoots and eventually died. Higher doses of radiation resulted in a decrease in leaf width, leaf length and root length to a statistically significant difference (Table 2). This was consistent with the findings of Pongchawee *et al.* (2007), who found that *A. congensis* N.E. Brown raised *in vitro* and exposed to gamma radiation at a dose of 40 to 120 Gy did not develop any new roots. It would be possible that high doses of radiation could destroy the enzymes necessary for the synthesis of IAA from tryptophan, since IAA promotes root growth. Plants exposed to high doses of radiation could not develop new roots (Gunckel, 1957). A likely cause is that radiation affects organic molecules that are essential to the cell division process, causing cell division to stop. If radiation damages the genetic material of the cells then the nucleic acid transcription process cannot proceed normally, and the organic molecules necessary for cell division may not be synthesized, leading to a halt in cell division and loss of viability (Neary *et al.,* 1957).

In the chronic irradiation experiment, it was found that plantlets exposed to radiation from a dose of 0 to 91.69 Gy did not have any statistically significant difference from the control with regard to the parameters of number of new shoots, number of new leaves, leaf width or root length. Samples that were exposed to radiation at a dose of 105.99 and 120.30 Gy showed a statistically significant difference decrease in the number of new shoots, number of new leaves, leaf length and leaf width compared to the control. However, at these levels of radiation, the number of new roots was higher than the control to a statistically significant difference (Table 3). It was possible that at this level of radiation the enzymes involved in the synthesis of IAA from tryptophan were stimulated, causing a promotion of root growth (Gunckel, 1957).

Mutations observed in the M_1V_3 generation

Following acute gamma irradiation at a dose of 20 Gy and higher, mutations observed in

Table 2 Mean ± standard deviation of number of roots, shoots, leaves, leaf length, leaf width and root length of *A. congensis* N.E. Brown in the M_1V_1 generation at 60 days after acute gamma irradiation.

Dose (Gy)	No. of roots	No. of shoots	No. of leaves	Leaf length (cm)	Leaf width (cm)	Root length (cm)
0	5.35±0.59a[1]	1.90±0.64a	5.30±0.80a	2.87±0.39a	0.76±0.09a	2.51±0.47a
20	5.70±0.73b	0.85±0.49b	2.80±1.54b	2.38±1.28a	0.50±0.28b	0.35±0.26b
40	0c	0.65±0.67b	2.45±1.54b	2.40±1.87a	0.50±0.39b	0c
60	0c	0.30±0.47c	0.55±0.89c	0.56±0.89b	0.26±0.41c	0c
80	0c	0c	0c	0b	0d	0c
100	0c	0c	0c	0b	0d	0c
F-test	*	*	*	*	*	*
C.V.(%)	8.95	6.68	10.59	1.84	2.64	14.56

* significant at 5% level.

1/ data within columns, means followed by a common letter are not significantly different at the 5% level by DMRT.

the M_1V_3 generation included narrower leaves, undulate leaf margins and dwarfism with light green leaves (Figure 2b-d; Table 4). These findings were consistent with the research of Tangsombatvichit (2008), who studied acute gamma radiation-induced mutations in cuttings of *Portulaca oleracea* L. and observed obviously apparent changes in the leaves and flower colors.

After chronic gamma irradiation, mutations observed in the M_1V_3 generation included dwarfism and albinism (Figure 2e-f; Table 5). This was similar to the findings of Wongpiyasatid *et al*. (2007) who observed changes in leaf color, leaf shape, flower color and canopy width of *Saintpaulia* (African violet) which were exposed to both acute and chronic gamma

Table 3 Mean± standard deviation of number of roots, shoots, leaves, leaf length, leaf width and root length of *A. congensis* N.E. Brown in the M_1V_1 generation at 60 days after chronic gamma irradiation.

Dose (Gy)	No. of roots	No. of shoots	No. of leaves	Leaf length (cm)	Leaf width (cm)	Root length (cm)
0	5.50±0.89a[1/]	2.50±0.61a	3.25±0.72a	2.85±0.39a	0.78±0.12a	2.62±0.48
14.34	16.05±0.85b	2.35±0.76a	3.55±0.61a	2.96±0.55ab	0.80±0.17a	2.67±0.53
28.60	15.95±1.19b	2.40±0.68a	3.45±0.69a	2.86±0.51a	0.89±0.50a	2.69±0.50
31.24	16.25±1.33b	2.30±0.73a	3.50±0.69a	2.92±0.56ab	0.83±0.18a	2.69±0.49
42.90	16.05±1.23b	2.50±0.89a	3.35±0.93a	3.17±0.46ab	0.83±0.17a	2.67±0.48
51.16	15.85±1.18b	2.45±0.60a	3.45±0.68a	3.24±0.50b	0.82±0.18a	2.76±0.52
65.55	15.90±1.29b	2.70±0.86a	3.55±0.60a	3.21±0.47b	0.84±0.21a	2.75±0.54
82.42	16.10±1.29b	2.55±0.94a	3.05±0.99a	3.17±0.38ab	0.84±0.22a	2.61±0.51
91.69	15.90±1.07b	2.40±0.75a	3.40±0.50a	3.20±0.53b	0.80±0.16a	2.72±0.52
105.99	30.30±4.05c	1.25±0.44b	1.90±0.55 b	1.32±0.37c	0.46±0.34b	2.89±0.47
120.30	34.10±4.64d	1.30±0.47 b	1.25±0.44c	1.18±0.32c	0.49±0.31b	2.87±0.50
F-test	*	*	*	*	*	ns
CV(%)	15.68	2.57	8.46	13.48	2.63	3.23

ns = non significant at 5% level.
* = significant at 5% level.
[1/] data within columns, means followed by a common letter are not significantly different at the 5% level by DMRT.

Table 4 Mutation frequencies in the M_1V_3 generation after acute gamma irradiation at 0-100 Gy and a dose rate equal to 264 Gy/hr. Mutants found were: dwarf plants, undulate leaf margins, narrow leaves and albinism.

Dose (Gy)	Total no. of plantlets	% Mutation	Mutant types (%)			
			Dwarf	Narrow leaves	Undulate leaf margins	Albino
0	60	0	0	0.	0	0
20	60	11.67	3.33	1.67	1.67	5.00
40	60	26.67	1.67	0	0	25.00
60	60	6.67	0	0	0	6.67
80	60	-	-	-	-	-
100	60	-	-	-	-	-

- = all plants died.

radiation. Similarly, Okamura *et al*. (2003) used gamma rays, X-rays and ion beams to induce mutations in *Dianthus* (carnation) *in vitro* and found many variations in flower color. In studying the effect of chronic gamma irradiation on the Bangkok Beauty variety of *Dracaena surculosa* Lindl., Ketmaro (2007) observed morphological changes in the leaves, which were probably due to changes in the genes that controlled leaf and plant morphology (Ichigawa *et al.,* 1970), resulting in phenotypic variations. Nevertheless, in this study the variegated leaves observed in the M_1V_1 generation were not transferred to the M_1V_3 generation. It is possible that the leaf color variegations in the M_1V_1 generation resulted from physiological damage rather than genetic damage. Leaf variegation like any other physiological damage, is unable to be passed on to the next generation through cell division. Therefore, in the M_1V_3 generation, leaf variegation disappeared. If the radiation treatment caused damage to a cell's nucleus or genetic material, then the observed variations could be inherited by the next generation (Lamseejan *et al.,* 2000).

A comparison of the gamma radiation doses administered as acute or chronic irradiation showed that the percentage of mutation differed greatly depending on the radiation dose rate. In particular, for doses of approximately 20-40 Gy, in this study the mutation frequencies for samples exposed to 20 and 40 Gy of acute irradiation were 11.67 and 26.67%, respectively, whereas the mutation frequencies for the samples exposed to 28.60 and 42.90 Gy of chronic radiation were 1.67 and 0%, respectively. This is probably because the radiation dose rate for the acute treatments was 264 Gy/hr while the radiation dose rate for the chronic treatments was only 0.71 Gy/hr. The lower dose rate of radiation probably caused little damage to the plants' genetic material so that the cells could repair themselves (Neary *et al.,* 1957) but the higher dose rate of radiation probably caused greater damage and in some cases resulted in death of the cells. As a result, in this study acute gamma irradiation resulted in a higher percentage of mutation. Moreover, radiation associated with *in vitro* culture could induce mutation in a short time, giving novel characters. If the mutations were interesting and attractive they may have high market value. Dwarf varieties of *A. congensis* N.E.

Table 5 Mutation frequencies in the M_1V_3 generation after chronic gamma irradiation at 0-120 Gy and a dose rate equal to 0.71 Gy/hr. Mutants found were: dwarf plants, narrow leaves and albinism.

Dose (Gy)	Total no. of plantlets	% Mutation	Mutant types (%)		
			Dwarf	Narrow leaves	Albino
0	60	0	0	0	0
14.34	60	1.67	0	0	1.67
28.60	60	1.67	1.67	0	0
31.24	60	3.33	0	0	3.33
42.90	60	0	0	0	0
51.16	60	0	0	0	0
65.55	60	0	0	0	0
82.42	60	0	0	0	0
91.69	60	5.00	0	0	5.00
105.99	60	11.67	0	0	11.67
120.30	60	10.00	0	0	10.00

Brown could be very popular with owners of small aquariums, which could open up a new customer group. If market demand for the mutated varieties was high they could be reproduced quickly using tissue culture.

The potential mutants of *A. congensis* N.E. Brown in this research were the undulate leaf margins (Figure 2b), dwarfism and light green leaves (Figure 2d) and dwarfism (Figure 2e).

CONCLUSION

The most appropriate amount of acute gamma irradiation for inducing mutations in *A. congensis* N.E. Brown was 20-30 Gy (a dose rate of 264Gy/hr), based on the $LD_{50(60)}$. Plants that were exposed to 80 Gy of radiation or more ceased to grow and eventually died. Variations were observed in the M_1V_3 generations (20 Gy) in the

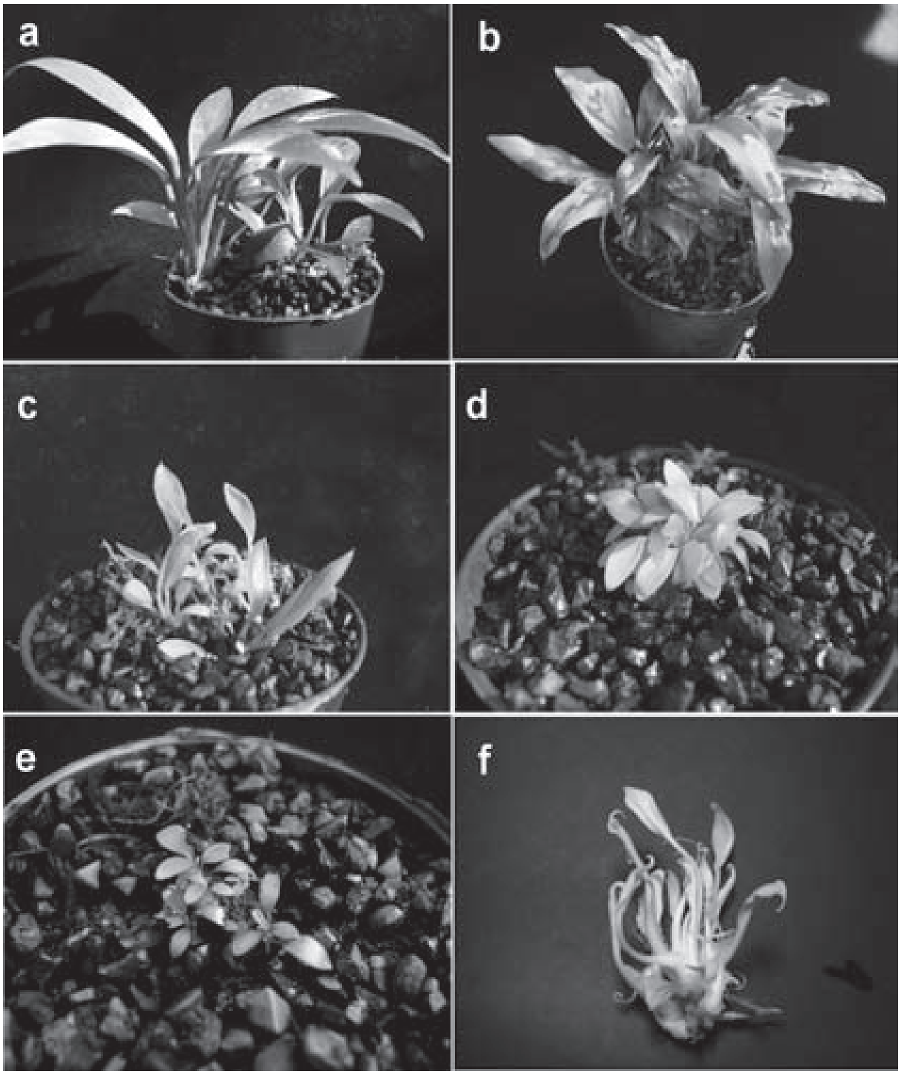

Figure 2 (a) *A. congensis* N.E. Brown at 3 months (control), (b) *A. congensis* N.E. Brown mutant undulate leaf margins, (c) narrower leaves and dwarfism, (d) dwarfism and light green leaves following acute gamma radiation at a dose of 20 Gy. (e) *A. congensis* N.E. Brown mutant dwarfism and (f) albino following chronic gamma radiation at a level of 28.60 Gy.

form and shape of the leaves, the leaf color and dwarf plants. On the other hand, when *A. congensis* N.E. Brown was exposed to chronic gamma radiation at 2 m (a dose rate of 0.71 Gy/hr), the survival rate was 100% for all treatments with low mutation frequency. The acute gamma irradiation resulted in a higher percentage of mutation than chronic irradiation because of a dose rate effect. Three mutant lines were obtained from this research.

ACKNOWLEDGEMENT

This research was financially supported by Kasetsart University Research and Development Institute (KURDI) and the Graduate School, Kasetsart University

LITERATURE CITED

Cuany, R.L., A.H. Sparrow and V. Pond. 1985. Genetic response of *Antirrhinum majus* to acute and chronic plant irradiation. **Molecular and General Genetics MGG** 89: 1432-1874.

Gunckel, J.E. 1957. The effect of ionizing radiation on plants: morphological effects. **Q.Rev.Biol.** 32: 46-56.

Ichikawa, S., K. Yamakawa, F. Sekiguchi and T. Tatsuno. 1970. Variation in somatic chromosome number found in radiation induced mutation in *Chrysanthemum morifolium* Hemsl. Cv. Yellow Delaware. **Radiat. Bot.** 10: 557-562.

Jompuk, P., S. Lamseejan and S. Deeseepan. 2001. The Induction of Mutations in Chrysanthemum Using Gamma rays and *In Vitro* Culture Techniques (In Thai, English abstract). pp. 15-24. *In* **Proceeding of the 8th Nuclear Science and Technology Conference.** 20-21 June 2000, Bangkok, Thailand.

Ketmaro, S. 2007. **Improvement of Dracaena (*Dracaena godseffiana*) Varieties by Using Gamma Rays**. M.S. Thesis. Kasetsart University. Bangkok.

Klein, R.M. and D.T. Klein. 1971. Post-irradiation modulation of ionizing radiation damage to plants. **The Botanical Review** 37: 397-436.

Lamseejan, S., P. Jompuk, A. Wongpiyasatid, S. Deeseepan and P. Kwanthamachart. 2000. Gamma-rays induced morphological changes in chrysanthemum (*Chrysanthemum morifolium*). **Kasetsart J. (Nat. Sci.).** 34(3): 417-422.

Masami, W. 2002. Molecular Mechanism of Cell Death by Radiation. **Nippon acta radiologica.** 62: 540-544.

Mühlberg, H. 1982. **The Complete Guide to Water Plants**. Sterling Publishing Co., Inc., German Democratic Republic. 392 p.

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

Neary, G.J., H.J. Evans and S.M. Tonkinson. 1957. Mitotic Delay Induced by Gamma Radiation in Broad Bean Root Meristems. **Nature** 179: 917-918.

Okamura, M., N. Yasuno, M. Ohtsuka, A. Tanaka, N. Shikazono and Y. Hase. 2003. Wide variety of flower-color and shape mutants regenerated from leaf cultures irradiated with ion beams. **Nucl. Instrum. Methods Phys. Res. Sect. B: Beam Interact. Mater. Atom.** 206: 574-578.

Pongchawee, K., R. Pradissan and W. Pipatcharoenchai. 2007. Induce mutation in *Anubias* spp. through *in vitro* Irradiation. **Thai Fisheries Gazette** 60(6): 493-497

Rataj, K. and T.J. Horeman. 1977. **Aquarium Plant: Their Identification, Cultivation and Ecology**. T. F. H. Publ. Inc., West Sulvania. 448 p.

Seneviratne, K.A.C.N. and D.S.A. Wijesundara. 2007. First African Violets (*Saintpaulia ionantha*, H. Wendl.) With a Changing Colour Pattern Induced by Mutation. **Am. J. Plant**

Physiol. 2(3): 233-236.

Tangsombatvitchit, C., A. Wongpiyasatid, P. Jompuk and T. Taychasinpitak. 2008. Effects of Acute Gamma Irradiation on Mutation from Stem Cuttings of *Portulaca oleracea* L.. **Agricultural Sci. J.** 39(1): 55-64.

Wang, Y., F. Wang, H. Zhai and Q. Liu. 2006. Production of useful mutant by chronic irradiation in sweetpotato. **Scientia Horticulture** 111: 173-178.

Wongpiyasatid, A., T. Thinnok, T. Taychasinpitak, P. Jompuk, K. Chusreeaeom and S. Lamseejan. 2007. Effects of acute gamma irradiation on adventitious plantlet regeneration and mutation from leaf cuttings of african violet (*Saintpaulia ionantha*). **Kasetsart J. (Nat. Sci.)**. 41(4): 633-640.