Effect of Gamma-ray Radiation on Morphological Development of *Orthosiphon stamineus* (Cat Whisker)

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Abstract: In this study, the vegetative parts of *Orthosiphon stamineus* were exposed to gamma radiation with 3, 6, 9 and 12 minutes exposure time period by using gamma cell 220 Co-60 machines at 0.08344 Gy dosages to investigate the effect of gamma ray. After 7 weeks of gamma ray radiation, plant survival percentages, height of plants, number of leaves, chlorophyll and carotenoid content were recorded and calculated every week. Survival rates of gamma ray irradiated explants of *Orthosiphon stamineus* were changed dramatically. It was found that the height of plant and number of leaves per plant was decreased following irradiation. Gamma ray irradiation also inhibited chlorophyll and carotenoid content compared to non-irradiated plants which showed higher content of chlorophyll and carotenoid.

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1. Introduction

Orthosiphon stamineus Benth known as Misai kucing and Kumis kucing in Malaysia. This herb belongs to *lamiaceae* or *labiatae* family, which is also known as the mint family. *O. stamineus* is widely grown in Southeast Asia and the tropical countries. Leaves of this plant are used as herbal tea and known as "Java tea" in some of Asian and European countries. Gamma rays are often used on plants in developing varieties that are agriculturally and economically important and have high productivity potential (Jain *et al.,* 1998). Gamma rays are also very important in mutation breeding and in *in vitro* mutagenesis in order to develop required features of plants and increase the genetic variability. Gamma irradiation can be useful for the alteration of one or a few physiological characters (Lapins, 1983). Mutagenesis by means of gamma rays has played an important role in the producing new mutants with improved properties which can produce higher amounts of commercially important metabolites (Sanada, 1986). Gamma rays with different irradiation levels can damage or modify important components of plant cells and even have been reported to affect differentially the morphology, anatomy, biochemistry and physiology of plants (Wi *et al*., 2007; Kovacs & Keresztes, 2002).

Irradiation-induced mutation breeding is effective in improving sweet potato characters such as yield, starch and soluble sugar content, carotenoids content of storage roots and disease resistance (Kukimura, 1986; Wang *et al*., 2007). Irradiation has also been successfully used for mutation breeding in various crops and ornamental plants (Song & Kang, 2003) and has proven encouraging the expression of recessive genes and producing new genetic variations (Schum, 2003; Song & Kang, 2003; Yoon *et al*., 1990). Shin *et al.* (2011) reported sweet potato mutants by irradiation produced high yield and high starch content. Biochemical studies on *O. stamineus* revealed that the total soluble protein and total chlorophyll content decreased notably as the gamma dosage increases (Kiong *et al.,* 2008). They also observed severe increase in specific activity of peroxidase especially in plantlets irradiated at high gamma ray dosages. The present study was conducted to investigate the effect of gamma irradiation with different exposure times. The dosage rate and suitable exposure time to produce *Orthosiphon Stamineus* mutant with commercial value was determined.

2. Materials and Methods

The composition of growth medium for *Orthosiphon Stamineus* was clay and organic soil with 2:1ratio. The healthy, young and green colour stems of *O.stamineus* were obtained from *in vivo* grown plants at Centre of Foundation Studies in Science, University of Malaya, Malaysia. Each stem cuts contain 3-4 internodes, 5 buds and 2-3 leaves. They were cut horizontally at about 10-15 cm each with leaves due to undergo photosynthesis during their growth and development. They were cleaned and washed to reduce contamination and were submerged in distilled water for 1 hour. For each exposure time, 10 stem cuts were prepared; hence 50 buds were radiated for each irradiating time. In this experiment, 5 stem cuts were placed inside a Schott Bottle as container and the lid was covered by aluminium foil. Radiation procedure: Gammacel-220 was used as source of Gamma ray with 0.08344 Gy*. The Scott bottles containing the samples were located inside the sample chamber. The sample containers were irradiated separately with gamma rays with four different exposure times (3, 6, 9 and 12 minutes). After 24 hours, the samples were taken out and were planted into prepared soil medium.

2.1. Extraction and determination of chlorophyll and carotenoid content

Green, healthy and young leaves from different radiation time and non-irradiated plants were taken after 7 weeks of plant gamma ray *in vivo*. Every sample was weighed out at approximately 0.2 g in the dark and cold conditions and was ground by chilled mortar and pestle containing 10 ml 80% (v/v) aqueous acetone with 2 drops of $MgCO_3$. All the chlorophyll extracts were put into the centrifuge tube and was wrapped by aluminium foil to prevent exposure to light and were kept in ice container. The clear supernatant after 5 minutes centrifugation were analysed for chlorophyll a and b concentrations by using spectrophotometer at the wavelength of 645, 652 and 663 nm. Chlorophyll and carotenoid content were calculated based on Lichtenthaler (1987).

3. Results and Discussions

Survival rates of irradiated explants remarkably decreased with the increase in time of exposure to gamma irradiation (Fig.1). High dosage of gamma radiation might increase plant sensitivity to gamma rays by reducing amount of endogenous growth regulators as a result of breakdown or lack of synthesis due to radiation (Lea, 1947; Kim *et al*., 2004). The average height of plants decreased with the increase in gamma radiation time (Fig. 2). Non- irradiated plants produced the highest average numbers of leaves (12.3±8.8) compared to 3,6,9 and 12 minutes radiated plants, which are 11.5±6.6, 6.1±4.4, 0.9±2.1and 0.3±0.9, respectively (Table 1). All irradiated plants exhibited lower amount of total chlorophyll content (TCC) compared to control plants (Fig. 3). Chlorophyll a content is higher than chlorophyll b content in irradiated and non-irradiated plants. This amount for non-irradiated plant is 42.3% and irradiated plants at 9 minutes showed the highest percentage of chlorophyll a content against chlorophyll b content (Fig. 4). An increase in radiation time caused a reduction in carotenoid content. The irradiated plants showed lower amount of carotenoid content compared to control plants. A remarkable decline of carotenoid content was observed in plants irradiated at 9 minutes of radiation time.

Table 1. Gamma ray effect on average number of leaves

Trait	Control	Gamma irradiation time (min)			
		3	6	9	12
Average number of leaves	12.3±8.8	11.5±6.6	6.1±4.4	0.9±2	0.3±0.9

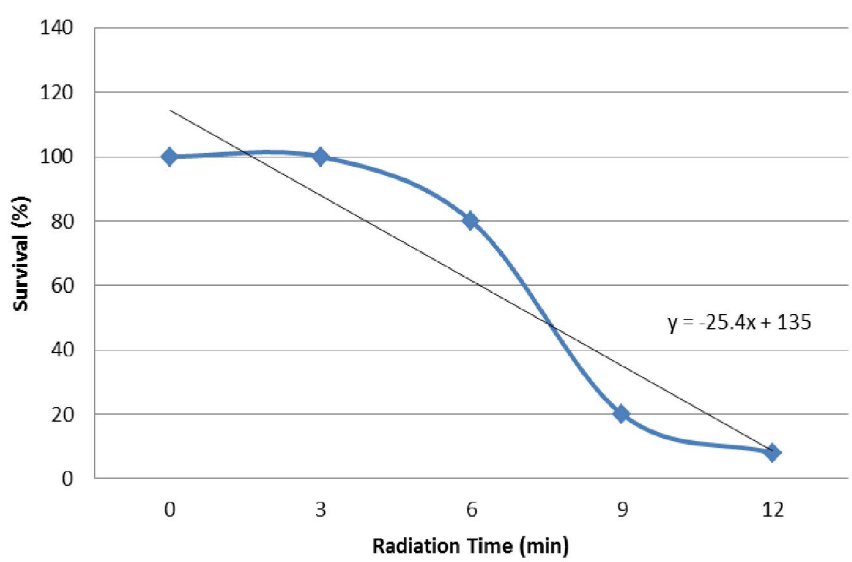

Figure 1. Percentage of survival of *O.stamineus* after 4 weeks of gamma irradiation

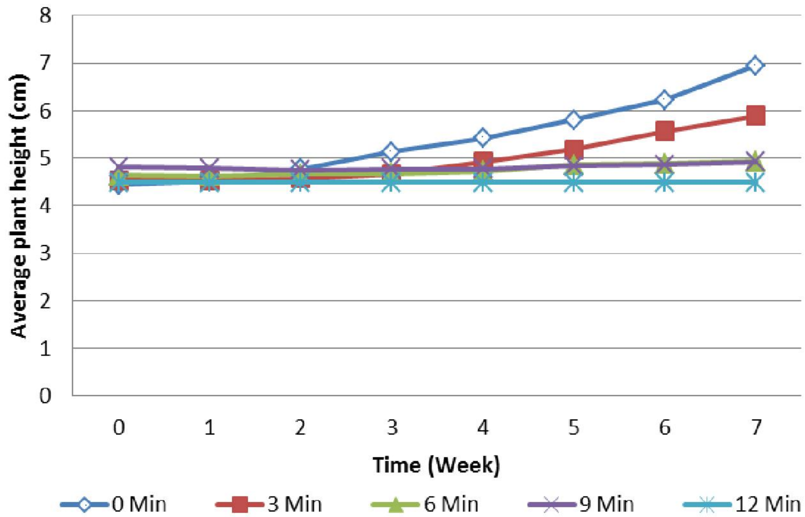

Figure 2. Growth rate of *O. stamineus* for 7 weeks

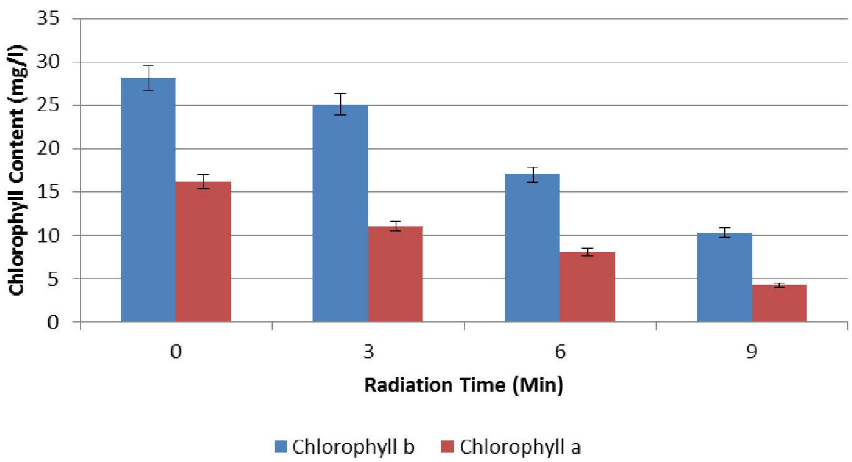

Figure 3. Effect of gamma irradiation on chlorophyll a and b content of *O.stamineus*

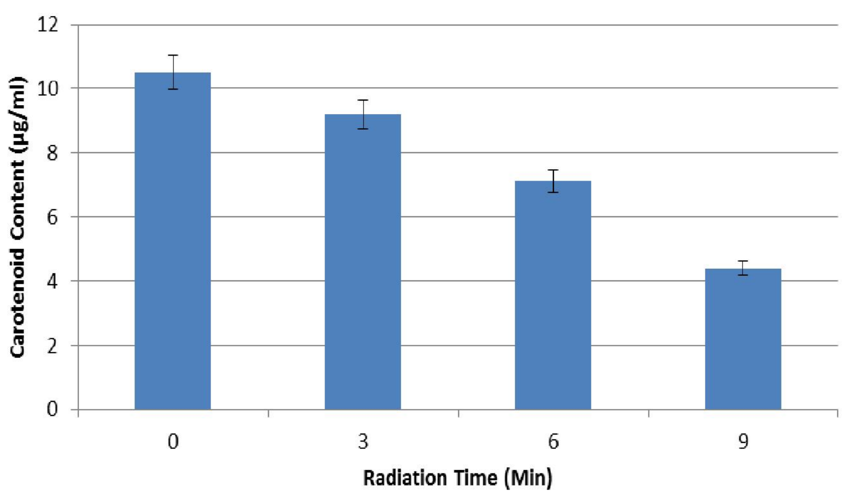

Figure 4. Effect of gamma irradiation on carotenoid content of *O.stamineus*

* The amount of energy absorbed per unit weight of the organ or tissue is called absorbed dose and is expressed in units of gray (Gy)

Figure 5. Normal and abnormal leaves shape of *Orthosiphon Stamineus* Benth

A) Normal leaves (Control)
B) Crescent shape leaf observed on 3 minutes radiated plants
C) Twin shape leaf on 6 minutes radiated plants
D) Twin shape leaf on 3 minutes radiated plants

Figure 6. *O. stamineus* Benth, 7 weeks after planting from left, control, 3, 6, 9 and 12 minutes exposed to gamma ray

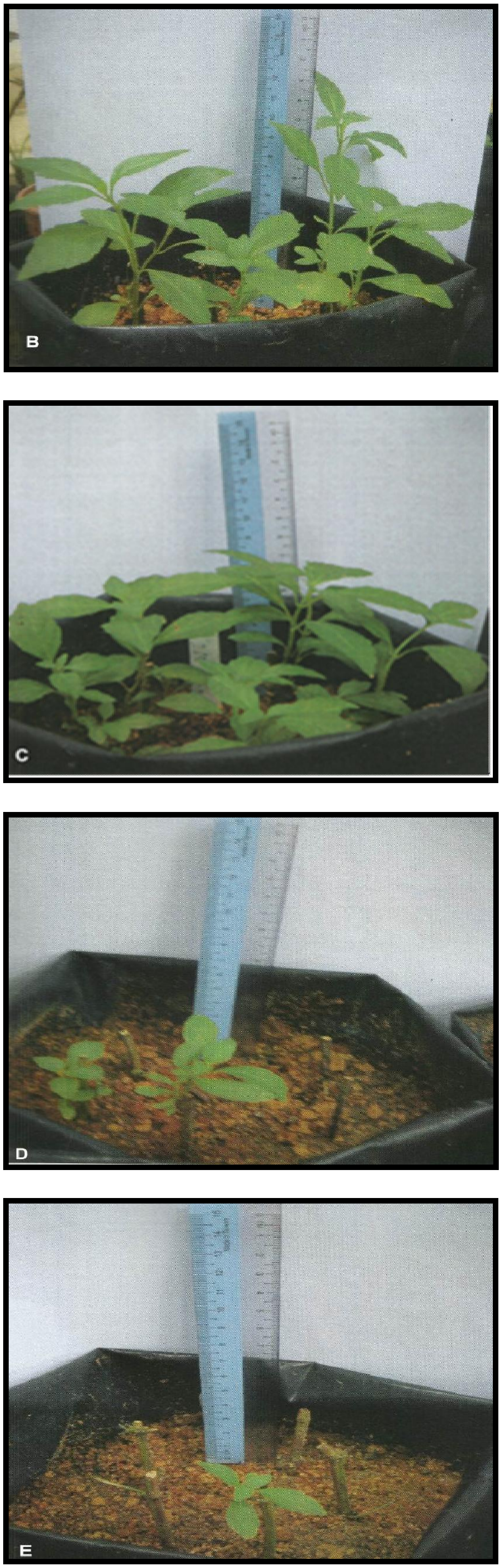

Figure 7. *O. stamineus* at 0 (B), 3 (C), 6 (D), 9 (E) and 12 (F) minutes exposed to gamma ray

4. Conclusions

Earlier, it was reported that total chlorophyll and carotenoid content were reduced in radiated plants by gamma ray (Kumar *et al*., 2009; Mathur, 1989; Borzouei *et al*., 2010; Simkin *et al*., 2003; Pawar *et al*., 2010). This research was successful in inducing mutation in *Orthosiphon stamineus* plants in exposure to gamma ray. The exposure time and applied dosage can be used to produce mutant plants in future. To make the treatment more efficient, repetitive treatment on the mutated plant parts and treatment to plants growing *in vitro* is suggested.

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References

1. Lichtenthaler HK, Chlorophylls and carotenoids: Pigments of photosynthetic biomembrane. Methods in Enzymology, 1987; 148: 350-382.
2. Borzouei A, Kafi M, Khazaei H, Naseriyan B, Majdabadi A. Effects of Gamma Radiation on Germination and Physiological Aspects of Wheat (*Triticum Aestivum* L.) Seedlings. Pakistan Journal of Botany, 2010; 42(4): 2281-2290.
3. Jain SM, Ahloowalia BS, Veilleux RE. Somaclonal Variation and Induced Mutation in

Crop Improvement. Kluwer Academic Publishers, 1998; 12(3): 203-218.
4. Kim JH, Baek MH, Chung BY, Wi SG, Kim JS. Alterations in the photosynthic pigments and antioxidant machineries of red pepper (*Capsicum annum* L.) seedling from gamma irradiated seeds. Plant Bioogy, 2004; 47: 314-321.
5. Kiong A, Ling Pick A, Grace Lai SH, Harun AR. Physiological responses of orthosiphon stamineus plantlets to gamma irradiation. Am-Eurasian J. Sustain Agric, 2008; 2(2): 135-149.
6. Kovacs E, Keresztes A. Effect of gamma and UV-B/C radiation on plant cell. Micron, 2002; 33: 199–210.
7. Kukimura H, Mutation breeding in sweet potato and tuber crops. Gamma Field Symp, 1986; 25: 109-130.
8. Kumar A, Parmhansh P, Prasad R. Induced Chlorophyll and Morphological Mutations in *Mungbean Vigna Radiata* L. Wilczek Department of Plant Breeding and Genetics, Rajendra Agricultural University, Pusa (Samastipur)-848 125, India Legum. Indianjournals, 2009; 32(1): 41-45.
9. Lapins T, Mutation breeding: methods in fruit breeding. Gamma Field Symposia, 1983; 1(3): 74-99.
10. Lea ED, Action of radiation on living cells. London: Cambridge University Press, 1947.
11. Mathur A. Mutation studies in buckwheat (Fagopyrum) II. Gamma ray induced chlorophyll mutations and vital mutation frequencies. Fagopyrum, 1989; 9: 15-22.
12. Pawar N, Pai S, Nimbalkar M, Kolar F, Dixit G. Induction of chlorophyll mutants in *Zingiber officinale* Roscoe by Gamma Rays and MS. Emirat Journal Food Agriculture, 2010; 22(5): 406-411.
13. Sanada T. Induced mutation breeding in fruit trees resistant mutant to black spot disease of Japanese pear. Gamma Field Symposia, 1986; 25(4): 87-108.
14. Schum A. Mutation breeding in ornamentals and efficient breeding method. Acta Hortica, 2003; 612: 47-60.
15. Shin JM, Kim BK, Seo SG, Jeon SB, Kim JS, Jun BK et al. Mutation breeding of sweet potato by gamma-ray radiation. African Journal of Agricultural Research, 2011; 6(6): 1447-1454.
16. Simkin AJ, Laboure AM, Kuntz M, Sandmann G. Comparison of Carotenoid Content, Gene Expression and Enzyme Levels in Tomato (Lycopersicon esculentum) Leaves. Verlag der Zeitschrift für Naturforschung, 2003; 58(C): 371-380.
17. Song H, Kang S. Application of Natural Variation and Induced Mutation in Breeding and Functional Genomics: Papers for International Symposium; Current Status and Future of Plant Mutation Breeding. Korean Journal of Breed Science, 2003; 35(1): 24-34.
18. Wang Y, Wang F, Zhai H, Liu Q. Production of a useful mutant by chronic irradiation in sweet potato. Science Hortica, 2007; 111(2): 173-178.
19. Wi SG, Chung BY, Kim, JS, Kim JH, Baek MH, Lee JW, Kim YS. Effects of gamma irradiation on morphological changes and biological responses in plants. Micron, 2007; 38(1): 553-564.
20. Yoon K, Park Y, Im B. Effect of gamma radiation on seed germination and androgenesis in Nicotiana tabacum L. Korean Journal of Breed Science, 1990; 21: 256-262.

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