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

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Abstract. The vegetative parts (leaves and stems) of *Orthosiphon stamineus* were exposed to gamma radiation with 3, 6, 9 and 12 minutes exposure period by using gamma cell 220 Co-60 machines at 0.08344 Gy dosages for investigation effect of gamma ray on this plant. After 7 weeks of gamma ray radiation and planting, plant survival percentages, height of plants, number of leaves, leaves morphology; chlorophyll and carotenoid content were recorded and calculated every week. The non-irradiated plants treated as control and it was found that 3 minutes gamma ray radiated plants showed the highest percentages, 100 and 80% of survival rates, the highest average number of leaves, 12.3 ± 8.8 and 11.5 ± 6.6 and plant height of 2.51 and 1.35 cm, compared to other plants, respectively. Non-irradiated plants showed higher content of chlorophyll and carotenoid compared to irradiated plants.

Keywords: gamma ray, *Orthosiphon stamineus*, morphology, chlorophyll, carotenoid

1. Introduction

Gamma rays with different irradiation levels can damage or modify important components of plant cells and even have been reported to affect differently the morphology, anatomy, biochemistry and physiology of plants [1], [2].

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 [3], [4]. Irradiation has also been successfully used for mutation breeding in various crops and ornamental plants [5]-[7]. This has proven encouraging the expression of recessive genes and developing new genetic variations [8]-[10]. Reference [11] reported mutant plants by irradiation produced high yield and high starch content.

O.stamineus Benth is 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.

Orthosiphon stamineus is widely grown in Southeast Asia and the tropical countries. The leaves of this plant are used as herbal tea and are known as “Java tea” in some of Asian and European countries.

Reference [12] reported bioactive pentacyclic triterpenes betulinic acid, oleanolic acid, ursolic acid and b-sitosterol from the leaves of this plant. More than twenty phenolic compounds were identified and quantified by HPLC from this plant [13]. There are some reports for strong antioxidant [14] and anti-microbial compounds [15], [16] of methanol extract of this plant. In addition, the fever-reducing efficacy of *O. stamineus* was comparable with that of acetaminophen [17].

The present study was conducted to examine and investigate the morphology of plant and the concentration of chlorophyll and carotenoid of the plant after irradiation with different exposure times of

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gamma ray. The dosage rate and suitable exposure time to produce *O.stamineus* mutant with commercial value was determined.

2. Materials and Methods

The composition of growth medium for *O.stamineus* was clay and organic soil with 2:1 ratio.

2.1 Plant material

The healthy, young and green colour stems of *O.stamineus* were obtained from *in vivo* grown plants in Pusat Asasi Sains, 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 the container and the lid was covered with aluminium foil.

2.2 Radiation procedure

Gammacel-220 was used as a source of Gamma ray with 0.08344 Gy. The Scott bottles containing the samples were located inside the sample chamber.

With four different exposure times (3, 6, 9 and 12 minutes) with gamma ray. The sample containers were irradiated separately.

After 24 hours, the samples were taken out and were planted into the prepared soil medium.

2.3 Chlorophyll and carotenoid contents

Green, healthy and young leaves from different radiation time and non-irradiated plants were taken after 7 weeks planting *in vivo*. Every sample was weighed out at approximately 0.2 g in the dark and cold conditions and was grounded 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, was wrapped with aluminium foil to prevent exposure to light, and were kept in ice container. The clear supernatant after 5-minute centrifugation were analysed for chlorophyll a and b concentrations by using a spectrophotometer at the wavelength of 645, 652 and 663 nm. Chlorophyll and carotenoid content were calculated based on [18].

3. Results

Survival rates of irradiated plants remarkably dropped with increase in time of exposure to gamma radiation (Fig. 1).

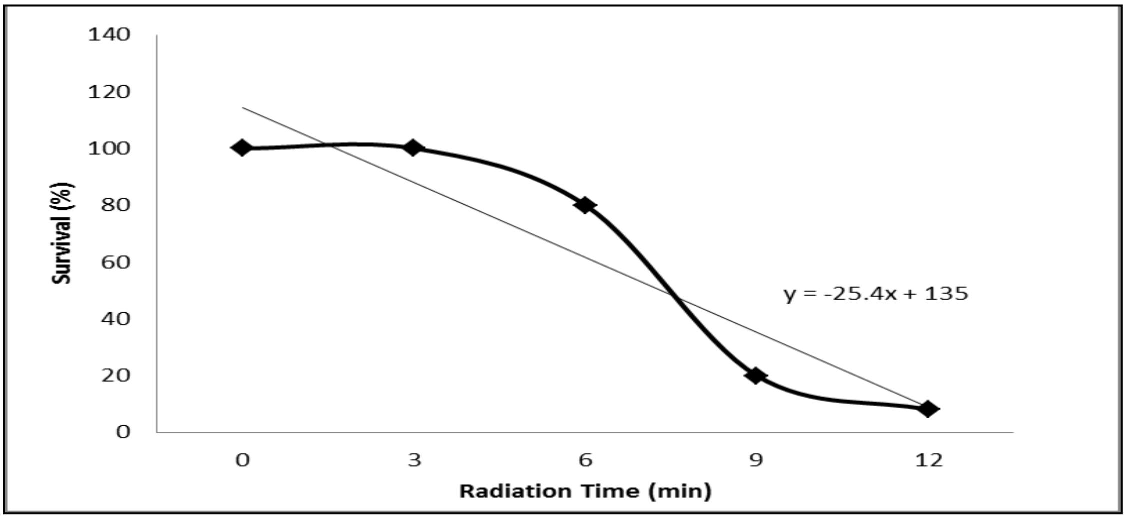

Fig. 1: Percentage of survival of *O. stamineus* after 4 weeks of exposure to gamma radiation

The high dosage of gamma radiation might increase plant sensitivity to gamma rays by reducing the amount of endogenous growth regulators because of breakdown or lack of synthesis due to radiation [19], [20].

As the result shown in Fig. 2, the plants average height was decreased with increasing radiation time.

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±2.1 and 0.3±0.9, respectively.

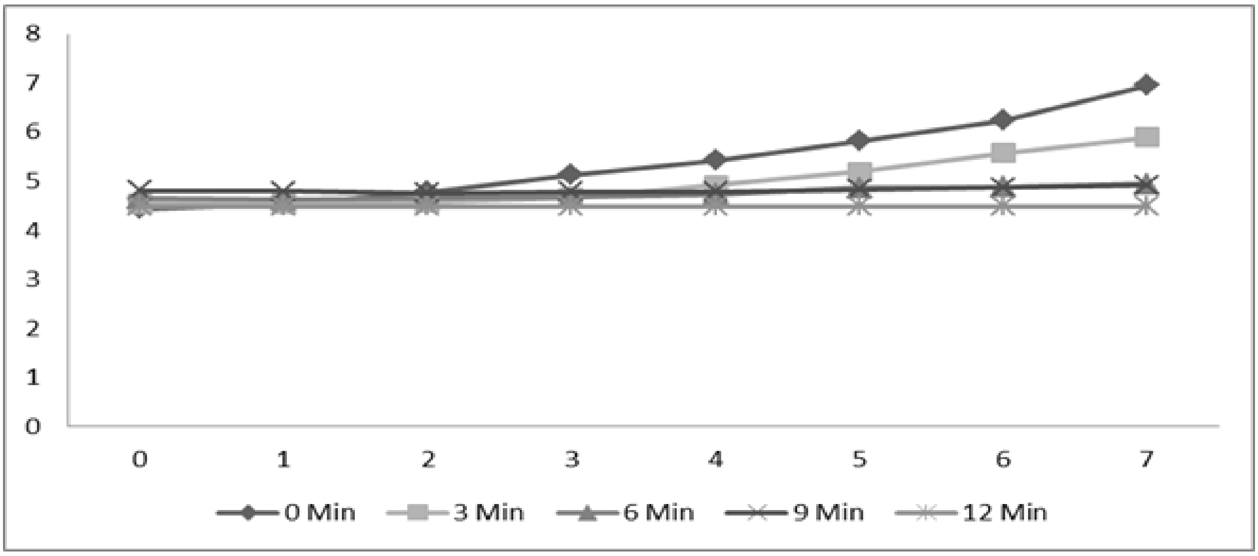

Fig. 1: Growth rate of *O. stamineus* after 7 weeks

The all irradiated plants exhibited lower amount of total chlorophyll content (TCC) compared to control plants.

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 against chlorophyll b (Fig. 3).

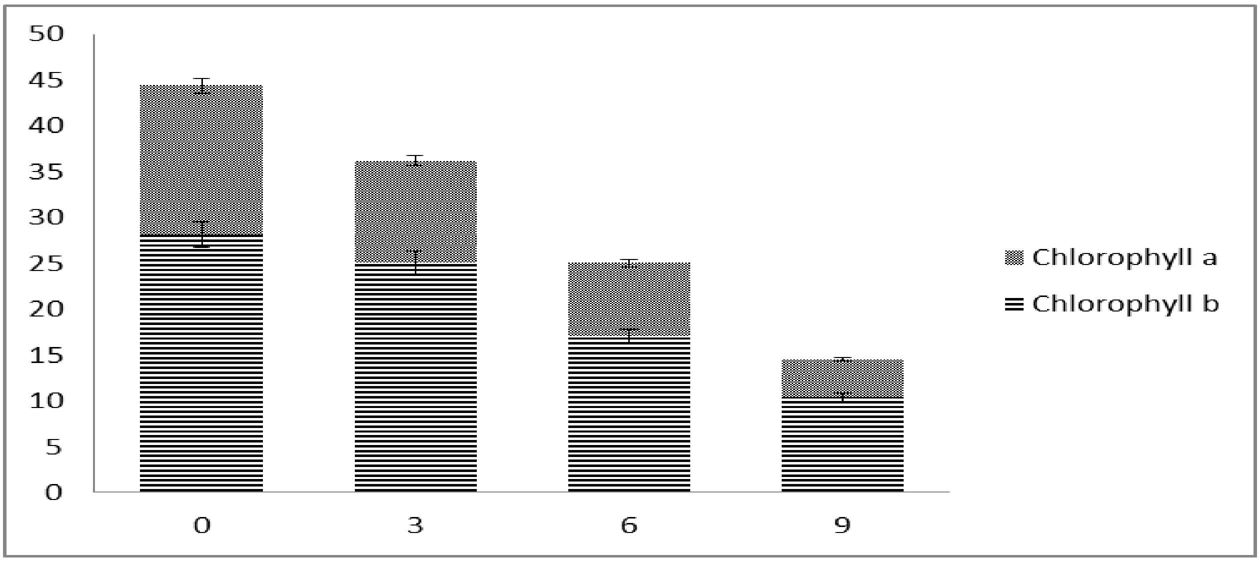

Fig. 2: Effect of gamma irradiation on chlorophyll a and b content of *O. stamineus*

An increase in radiation time caused a reduction in carotenoid contents. The irradiated plants showed the 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 (Fig. 4).

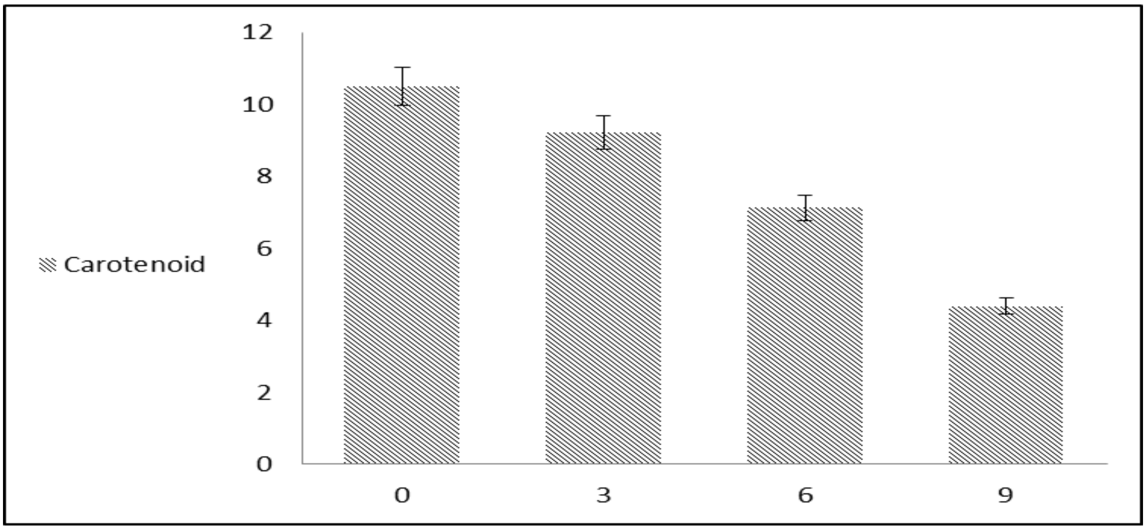

Fig. 4: Effect of gamma irradiation on the carotenoid content of *O. stamineus*

4. Conclusion

Earlier it was reported that total chlorophyll and carotenoid content were reduced in radiated plants by gamma ray [21]-[25].This research is successful in inducing mutation in *Orthosiphon stamineus* plant to gamma ray irradiation. 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 for plants growing *in vitro* is suggested

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