Effects of Gamma Radiation on Growth of *Sinningia speciosa* Callus Tissue

Kesan Radiasi Gamma kepada Pertumbuhan Tisu Kalus Sinningia speciosa

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Abstract

In this study, the *in vitro* tissue culture of the *Sinningia speciosa* callus was established at 25±1 ºC with light intensity 1000 µmol m^{-2} sec^{-1} and 70-80% relative humidity with a 16/8h light/dark photoperiod. Additionally, gamma radiation of 10-60 Gy was applied and the effects of gamma rays on growth of *Sinningia speciosa* callus tissue were investigated. A 100% callus initiation was observed in petal explants cultured on MS medium supplemented with combinations of 0.5 mg L^{-1} NAA and 2.0 mg L^{-1} BAP resulted in 100% callus induction after being cultured for 4 weeks. This medium was considered optimal medium for callus induction because it can produce normal growing healthy callus. Callus growth was based on callus weight and changing textures and colour of callus; determined following exposure of the calli to different doses of gamma rays. Results obtained indicated that callus growth was reduced when the dose of radiation was increased. It was found that increasing doses of gamma radiation (10-60 Gy) has inhibition effect on callus fresh weight on the 4-week established cultures of *Sinningia speciosa* callus. Gamma rays produced different effect compared to non-irradiated callus controls in morphological aspects of the callus such as textures and colour of callus cultures.

Keywords *Sinningia speciosa, gamma rays, callus growth.*

Abstrak

Di dalam kajian ini, kultur tisu *in vitro* kalus *Sinningia speciosa* telah dilakukan pada suhu 25±1 ºC dengan keamatan cahaya 1000 µmol m^{-2} sec^{-1} dan 70-80% kelembapan relatif dengan 16/8 jam foto kala cahaya/gelap. Sebagai tambahan, sinar gamma 10-60 Gy telah diberikan dan kesan sinar gamma ke atas pertumbuhan tisu kalus *Sinningia speciosa* telah dikaji. Sebanyak 100% inisiasi kalus diperhatikan pada eksplan kelopak yang dikulturkan di atas medium MS yang dibekalkan dengan kombinasi 0.5 mg L^{-1} NAA dan 2.0 mg L^{-1} BAP menghasilkan 100 % induksi kalus selepas dikulturkan selama 4 minggu. Medium ini dianggap sebagai medium optima untuk induksi kalus disebabkan ia boleh menghasilkan pertumbuhan kalus normal secara sihat. Pertumbuhan kalus ditentukan berdasarkan berat kalus, perubahan tekstur dan perubahan warna kalus setelah pendedahan kepada dos sinar gamma yang berbeza. Hasil kajian menunjukkan pertumbuhan menurun apabila dos radiasi ditingkatkan. Didapati peningkatan dos radiasi gamma (10-60 Gy) mempunyai kesan

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perencatan ke atas berat basah kalus setelah 4 minggu kultur kalus *Sinningia speciosa* dihasilkan. Sinar-sinar gamma memberikan kesan yang berbeza berbanding dengan kalus kawalan tanpa sinaran daripada aspek morfologi kalus seperti tekstur dan warna kultur kalus.

Kata kunci *Sinningia speciosa,* sinar gamma*,* pertumbuhan kalus*.*

Introduction

The Gloxinia (*Sinningia speciosa*) is a member of the indoor flowering plant family Gesneriaceae. The plants produce large, velvety and brightly coloured flowers. Gloxinia flowers come in colours ranging from pure white to pink, lavender, red, to deep purple. There are also flowers that come in two colours and those with white-border petals. The most popular cultivars in Malaysia are those in deep red and purple.

Induced mutation has been reported to be an efficient technique to achieve the desirable characters in flowers and ornamental plants (Maluszynski, 1995). In plant breeding, several methods may be used to increase the genetic variability within a crop. The main advantage of mutation induction in vegetatively propagated crops is the ability to change one or a few characters of an outstanding cultivar without altering the remaining and often unique part of the genotype.

Mutation breeding, on the other hand, makes use of the possibility of altering genes by exposing seeds or the other plant parts to chemical or physical mutagens. Mutations can be induced by chemicals, such as EMS (ethyl methanesulphonate), or various types of ionizing radiation (X-rays, neutrons, ultraviolet, gamma-rays, etc). There are reports on effects of gamma radiation on growth of callus tissue of crops and ornamental plants. An example of radiation-induced mutant of great commercial value is those of *Chrysanthemum morifolium* cv. Horim. The interest in the application of mutation induction in vegetatively propagated crops and ornamentals are a consequence of the large economic importance of many species of this group (Broertjes and Harten, 1978).

In an earlier research, Venketeswaran and Partanen (1966) had described the comparative study of the effect of gamma radiation on callus tissue of *Nicotiana* sp grown in suspension culture. They observed that low doses of gamma radiation slightly reduced the callus growth of *Nicotiana* sp. Broertjes and Harten (1978) proposed that *in vitro* induced mutation could be a very useful tool. By irradiation, the induced mutation could often affect the size of flower or/in a flower colour, but retained other desirable characteristics. Bajaj et al., (1970) have indicated the effects of gamma radiation on seeds, seedlings and callus cultures of *Phaseolus vulgaris*. They discovered irradiated seeds produced stunted seedlings, which later exhibited delayed flowering and poor seed set. However, when 8 days-old *Phaseolus vulgaris* seedling were irradiated, inhibition of growth occurred, followed by degeneration of the apical meristem and death, whereas, callus grown in suspension cultures showed significant stimulation of growth followed by a gradual decrease in growth (Bajaj et al*.,* 1970).

Spiegel-Roy and Kochba (1973) reported that gamma irradiation on ovular callus of *Citrus sinensis* caused a marked stimulation in embryoid formation. According to them, irradiation might cause a shift from conditions favaourable for callus growth to those favouring embryoid formation. According to Pandey et al. (1979), it was reported that gamma radiation at doses range between 800 to 2500 rads produced compact callus of *Haworthia mirabilis* as compared to the controls which were friable. Callus exposed to

800-2500 rads continued to grow with little or no organogenesis. Recently, Venkateshwarlu (2008) proposed a mutagenesis programme using physical mutagen (gamma rays) on *Cucumis melo* cv. Bathasa. The irradiated callus of *Cucumis melo* cv. Bathasa which was grown in solidified MS medium showed significant stimulation of callus growth at lower radiation doses, while at higher doses such as 15 and 20 kRs callus growth was drastically reduced.

Induced mutation breeding is an alternative strategy to conventional breeding. It holds promise for creating desired variability with gamma irradiation as the mutagenic agent. Substantial amount of work had been done on the effects of gamma irradiation on the whole plants, but reports on effect of irradiation on callus induction of ornamental plants were few. Hence, this study was attempted with the aim to study the effect of gamma radiation on callus growth of *Sinningia speciosa*. The morphological changes at different doses of gamma radiation were also observed.

Materials and Methods

Plant materials

All experiments were conducted at Laboratory B2.5, Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia. In these experiments, explants used were petals of *Sinningia speciosa* excised from 10 months-old intact plants. Excised petals were surface sterilized with Tween 20 and soaked in 100% Chlorox (Sodium hypochlorite) for 5 minutes and subsequently soaked with 50%, 30% and 20% Chlorox for 1 minutes, then rinsed three times with sterile distilled water in a laminar flow chamber. After the last washing, the explants were further cut into 5-10 mm pieces and ready to be cultured.

Preparation of induction callus medium and culture condition

After sterilization, the explants were cultured on MS medium (Murashige and Skoog, 1962) added with 8.0 g L^{-1} Oxoid agar (Sigma) and 30.0 g L^{-1} sucrose. The medium was also supplemented with combinations of 0.5 mg L^{-1} NAA and 2.0 mg L^{-1} BAP. The medium was autoclaved for 21 minutes at 121 ºC after the pH was adjusted to 5.8. The cultures were maintained in the culture room condition maintained at 25±1 ºC with light intensity 1000 µmol m^{-2} sec^{-1} and 70-80% relative humidity with a 16/8 h light/dark photoperiod.

Gamma Irradiation Treatments

For radiobiological studies, callus tissues were irradiated with ^{60}Cobalt gamma source (Model 220 Gammacell) at a dose rate of 0.216 Gy/sec (Gray/second). All experiments were conducted at Department of Physics, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia.

Harvesting

A total of 500 mg callus cultures were exposed to doses in the range of 0-60 Gy. Immediately after irradiation, the callus tissues were kept overnight in the dark under culture room

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condition. The callus tissues were then transferred and replaced to non-irradiated (fresh) medium of the same composition. The cultures were kept in the culture room maintained at 25±1 ºC with light intensity 1000 µmol m^{-2} sec^{-1} and 70-80% relative humidity with a 16/8 h light/dark photoperiod. At the end of 4-week culture period, total fresh weight and dry weight (70ºC oven dried for 48 hours) of the callus were measured and morphological changes of the callus tissues were observed and recorded. These experiments were repeated once and 25 cultures were used for each treatment.

Growth and percentage of survival

The measurement of growth rate was based on fresh weight measurement by weighing 25 tubes of each callus every week. Comparison between the average final fresh weights of irradiated callus tissues and the average initial fresh weight were determined after 4 weeks of culture. The growth rate was calculated as follows:

$$Growth\ Rate\ of\ Callus\ Cultures = \frac{Average\ \ fresh\ weight\ \ (Final) - Average\ \ fresh\ \ (Initial)}{Fresh\ weight\ \ (Initial)}$$

Water content

The fresh and dry weight of callus tissues of irradiated compared to the control was observed after 4-week culture period. The water content was measured by weighing 25 tubes of callus, and drying the tissue in hot air oven at 70ºC oven dried for 48 hours. The dried weight was measured and the water content, calculated. The water content was calculated as follows:

$$Water\ Content\ of\ Callus\ = \frac{Average\ \ fresh\ weight\ \ - Average\ dry\ \ weight}{Average\ \ Fresh\ weight}$$

Analysis data

All treatments consisted of 25 replicates and each replicate contained 500 mg of callus. The fresh weight, dry weight and growth rate were recorded after 4 weeks of cultures. The mean was calculated. Significant differences between treatment means were determined using one-way ANOVA.

Results and Discussion

Growth of Callus after Treated with Varying Doses of Gamma Radiation

In this study, petals were the most responsive explant to regenerate callus compared to leaf, peduncle and petiole of *Sinningia speciosa.* Petals have been used as explants in previous study on species of ornamental plant such as *Saintpaulia ionantha* (Daud, 2005). Nitsch and Nitsch (1967) mentioned that explants from reproductive parts have more potential in inducing flowering compared with explants from vegetative parts. Petal explants gave

100 % callus induction after 4 weeks in culture on MS medium containing 0.5 mg L^{-1} NAA and 2.0 mg L^{-1} BAP. This was considered as optimal medium for callus induction because it can produce fast growing healthy callus.

Callus tissue irradiated was grown on MS medium supplemented with 1.0 mg L^{-1} NAA and 2.0 mg L^{-1} BAP. Comparative differences in the radiobiological effects of gamma radiation on callus tissue cultures of *Sinningia speciosa* are shown in Table 1 and Figure 1. The growth rate of the callus cultures was measured within 4-week period or until the callus completely died.

The callus tissue irradiated at 10 Gy remained in creamy colour and the weights of irradiated callus were 1700.5 ± 0.3 (fresh weight) and 0.65 ± 0.2 (dry weight). The highest growth rate of calli was obtained at the lower dose of gamma ray 10 Gy which showed an increment as compared to the control (2.40 ± 0.3). However, reduction in calli fresh weight and dry weight were obtained in calli produced after treatments with 20 Gy, 30 Gy, 40 Gy, 50 Gy and 60 Gy. From these results, it is clear that the reduction in callus growth rate showed a gradual decrease with increasing gamma ray from 10 Gy to 60 Gy. At doses 60 Gy, the callus growth rate was 1.11 ± 0.2. This indicated that the callus fresh weight decreased when the dose of irradiation was increased (Table 1). Results (Table 1) show that there were significant differences (< 0.05) between the doses of gamma radiation application on callus fresh weight.

Table 1 Effects of varying doses of gamma radiation (10-60 Gy) on fresh weight and dry weight of callus tissue of *Sinningia speciosa* on MS medium containing 0.5 mg L^{-1} NAA and 2.0 mg L^{-1} BAP for 4 weeks. All cultures were kept at 25±1 ºC with 16 hours light and 8 hours dark photoperiod

Treatment Doses (Gy)	Replicate (number of vials)	Growth of Sinningia speciosa Callus Cultures [Initial Fresh Weight = 500 ± 0.0 mg]			
		Final Fresh Weight (mg)	Growth Rate (1)	Final Dry Weight (mg)	Water Content (2)
0 (Control)	25	1700.5 ± 9.0	2.40 ± 0.3	597.5 ± 9.5	0.65 ± 0.2
10	25	1590.1 ± 8.0	2.18 ± 0.3	590.0 ± 9.5	0.63 ± 0.4
20	25	1440.2 ± 8.5	1.88 ± 0.4	582.2 ± 8.0	0.60 ± 0.4
30	25	1284.0 ± 8.0	1.57 ± 0.5	577.0 ± 8.0	0.55 ± 0.4
40	25	1204.6 ± 8.0	1.41 ± 0.2	599.8 ± 9.5	0.50 ± 0.2
50	25	1140.5 ± 9.0	1.28 ± 0.2	605.2 ± 9.0	0.47 ± 0.2
60	25	1055.2 ± 9.0	1.11 ± 0.2	607.3 ± 9.0	0.42 ± 0.2

(1)

$$Growth\ Rate\ of\ Callus\ Cultures = \frac{Average\ \ fresh\ weight\ \ (Final) - Average\ \ fresh\ \ (Initial)}{Fresh\ weight\ \ (Initial)}\ ;\ after\ 4\ weeks\ of\ culture$$

(2)

$$Water\ Content\ of\ Callus\ = \frac{Average\ \ fresh\ weight\ \ - Average\ dry\ weight}{Average\ Fresh\ weight}$$

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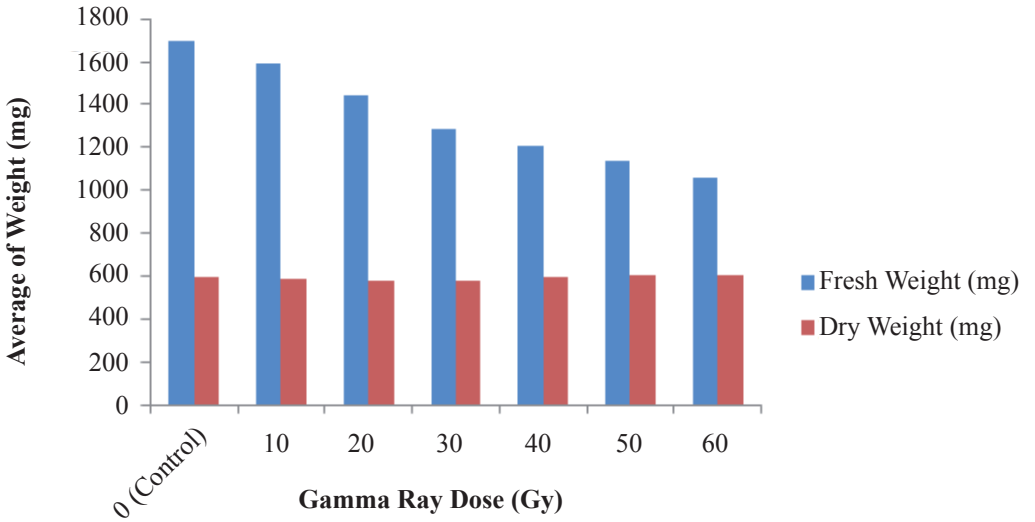

Figure 1 Comparison of the effect of varying doses of gamma radiation on fresh and dry weight of callus tissue for 4-week-culture period

Callus tissue subjected to varying doses of gamma radiation has yielded some interesting data on growth and radiosensitivity. There was a significant reduction in callus growth when doses of irradiation increased (Figure 2). A lot of studies have been done on effects of radiosensitivity in inhibiting of plant growth in various species. Similar decrease in callus fresh weight was observed in *Phaseolus vulgaris* (Bajaj et al., 1970), castor bean (Reddy et al., 1987), and *Helianthus annus.L* (Omar et al., 1993) with the increase in the dose of irradiation. Pandey et al. (1979) also found similar type of decrement in callus

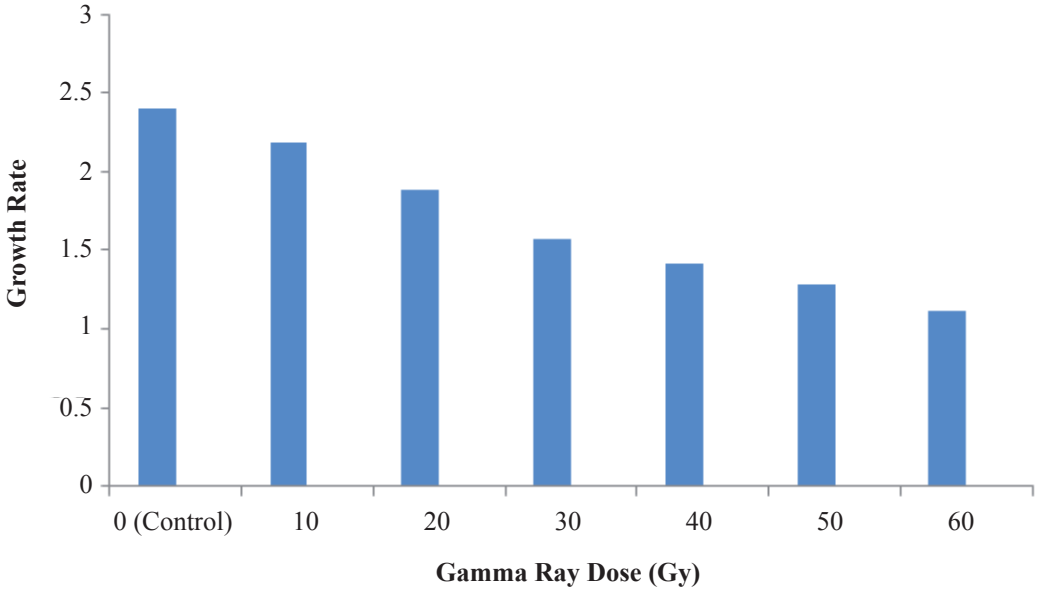

Figure 2 The effects of gamma radiation on callus growth rate

obtained with increased dose of gamma rays in *Haworthia mirabilis,* in which fresh and dry weights of callus receiving 1000 to 1500 rads exceeded those nonirradiated controls. Also, Abdel-Hady and Ali (2006) found that the reduction of callus growth rate in all studied wheat (*Triticum aestivum* L.) genotypes were due to the inhibiting effect of higher doses (350 Gy and 450 Gy). Venkateshwarlu (2008) reported there was a significant stimulation of growth in the irradiated callus at lower dose, while at higher doses (15 and 20 kRs) callus growth was drastically reduced. Hossain and Alam (2001) observed that at approximately 5.0 Gy of gamma radiation, a 50% inhibition of callus growth and plant regeneration was found in rice varieties. The reduction of growth caused by the reduced amount of endogenous growth regulators in the culture medium due to increased gamma ray dose.

Morphological Changes of Callus after Treated with Varying Doses of Gamma Radiation

Growth responses, colour and texture of the callus were influenced by the irradiation dose. The effects of varying doses of gamma irradiation on the morphological characters on callus tissue of *Sinningia speciosa* after 4 weeks in culture were observed (Table 2). At 10 Gy doses the callus produced had similar characters as compared to the nonirradiated controls. With increasing dose, the colour of the callus darkened and showed some necrotic effects. Callus cultures were slightly stimulated at 20 -30 Gy, later there was a decrease in growth at higher doses up to 60 Gy. Stimulation of growth at low doses of radiation has also been reported in other tissue as well (Bajaj et al*.,* 1970; Holsten et al*.,* 1965). In addition, compact callus was observed in all treatments (irradiated and nonirradiated callus).

Table 2 Effects of varying doses of gamma radiation on the morphological characters of callus tissue of *Sinningia speciosa* in MS medium containing 0.5 mg L^{-1} NAA and 2.0 mg L^{-1} BAP for 4 weeks. All cultures were kept at 25±1 ºC with 16 hours light and 8 hours dark photoperiod

Doses (Gy)	Morphological Charaters Observed
0 (Control)	produced normal growing healthy callus, compact callus with creamy in colour
10	produced normal growing healthy callus, compact callus with creamy colour.
20	slight stimulation of callus growth, produced compact callus with creamy to light brown colour
30	slight stimulation of callus growth, produced compact callus with creamy to light brown colour
40	gradual decrease in callus growth, produced compact callus with light brown to dark brown colour
50	gradual decrease in callus growth, callus showed some necrotic effects, produced compact callus and watery with dark brown colour
60	gradual decrease in callus growth, showed some necrotic effects produced compact callus and watery with dark brown colour

The damage caused by irradiation could be expressed at the metabolic level before they appear as growth retardation and death (Inoue et al., 1975). This might be attributed

to the fact that the extreme doses of gamma radiation induced drastic structural damage in chromosomes which could be harmful to the genotype for its desired combination of genes. In previous studies, exposure of high doses of gamma irradiation in bean callus cultures caused a decline in growth and inhibition of RNA and protein synthesis (Bajaj, 1970).

Conclusion

The investigation showed that irradiation affected the callus fresh and dry weights. An increasing doses of gamma radiation negatively affected the callus growth. Information gained from this study would further enhance the quality of the *in vitro* plantlets, which could possibly have an effect on the *Sinningia speciosa* plants under *in vivo* condition. Considering the frequency of regeneration through callus and the effect of irradiation, an alternative method for obtaining additional variants or increasing the genetic variability within indoor ornamental plant can be followed to generate large quantity of callus on specific media. Selective procedures to screen out variant cells, in attempts to identify useful genotypes (disease resistance and improved flower colour), can then be allowed to regenerate to give somaclonal variants.

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