CHAPTER 7

EFFECTS OF IRRADIATION ON CULTURES OF *Gerbera jamesonii* Bolus ex. Hook f.

7.1 EXPERIMENTAL AIMS

The methods of plant breeding have become increasingly sophisticated since the days of simple selection among natural populations, which consisted of mixtures of genotypes. Modern day plant breeding is based on creating variation, selection, evaluation and multiplication of desired genotypes. To increase efficiency and to cut short the time taken in each step, several techniques have been combined. Plant breeders have the options of using *in vitro* culture for rapid multiplication, molecular methods to select specific genotypes, mutagenesis to enhance variation, controlled environmental conditions to manipulate growth and flowering and many others.

Mutations induction could be done by either exposing explants to chemicals such as ethylmethanesulphate and colchicine or by physical mutagens such as irradiation using gamma and X-ray. The most preferred method to induce mutation breeding is by irradiation. Irradiation technique can influence the changes of plant phenotype such as plants heights and growths, flower colours and sizes as well as the patterns and colours of the leaves.

The use of nuclear techniques in plant breeding has been mostly directed for inducing mutations. Since the discovery of X-rays, the use of ionizing radiation such as

X-rays, gamma rays and neutrons for inducing variation has become an established technology. Induced mutations have been used in the improvement of major crops such as wheat, barley, rice, cotton, peanuts and beans which are seed propagated.

Many induced mutants have been released as cultivars and several others have been used as parents in the pedigree of some leading cultivars (Ahloowalia and Maluszynski, 2001). In vegetatively propagated plants, many mutants in ornamentals such as *Achimenes*, chrysanthemum, carnation, roses and *Streptocarpus* were obtained by irradiating rooted stem cuttings, detached leaves and dormant plants (Broertjes, 1977). The altered flower colour and shape, growth habit (dwarf or trailing) and other novel phenotype of commercial value were selected. According to the FAO/IAEA (Maluszynski *et al.*, 1992), of the 465 mutants release among the vegetatively propagated plants, most were floricultural plants and only a few fruit trees. These included chrysanthemums, *Alstroemeria*, dahlia, bougainvillea, rose, *Achimenes,* begonia, carnation, *Streptocarpus* and azalea Since the effect of mutation in ornamentals is very visible, selection for changed flower colour, shape and size is easy. Hence, mutation techniques have become a major tool for breeding ornamental plants (Maluszynski *et al.*, 1995).

The key factor in the irradiation of plant material is the dose, which is the amount of radiation energy absorbed by the material. The unit of measurement of radiation dose is Gray (Gy). Normally, high doses are used for sterilization purposes and low doses are used for mutations in plant materials.

The present work is carried out to study the effects of Gamma irradiation on the growth of explants *in vitro* and the formation of shoots and plantlets. Irradiation is known to exhibit or inhibit the differentiation of cells and growth of plants *in vitro*. Irradiation also helps in producing new plant varieties.

At the same time, the main objective of this chapter is to study the effect of gamma radiation on the organogenesis, callus formation and also development of *Gerbera* plantlets *in vitro*. Biochemical changes during post-irradiation were also studied. Changes in chlorophyll content and soluble proteins of irradiated *Gerbera* callus were analyzed. These experiments were carried out in order to compare the chlorophyll and total soluble protein content in irradiated and non-irradiated *G. jamesonii* callus. The maturation rate and flowering of *G. jamesonii* irradiated plantlets that were being transferred to the green house were also studied. It is hoped that the radiation effects could exhibit useful and applicable somaclonal variations in the morphological aspects of *G. jamesonii* plants compared to the natural mother plant. Hence, more economical varieties could be produced.

7.2 MATERIALS AND METHODS

7.2.1 Source of Explants

Gerbera jamesonii seeds were sterilized before being cultured in the culture medium (section 2.2.4). The seeds were cultured on basal MS medium with 30 g/l sucrose and 8.0 g/l technical agar for the growth of aseptic seedlings. Seeds were germinated *in vitro* after 6-7 days in culture. Complete plants were obtained after 6-8 weeks. Leaves and petiole from these seedlings were utilized as source of explants.

7.2.2 Source of Gamma Radiation

The gamma radiation was obtained from ^{60}Cobalt, 0026 Pool Irradiator with isotope model located at Physics Department, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia.

7.2.3 Gamma Radiation Dose

Gamma radiation dose used for this experiment was fixed at 10, 20, 30, 40, 50 and 60 Gray (Gy). The gamma dose was 0.204 Gy/second at the time this experiment was conducted. Therefore, each exposure of the gamma radiation was fixed at 49, 98, 147, 196, 245 and 294 seconds.

7.2.4 Effect of Gamma Radiation on Shoot Regeneration

In order to study the effect of gamma radiation on regeneration of shoots *in vitro,* petiole explants were used. The medium utilized was MS supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA. This medium was used since it is identified as the optimum medium for shoot regeneration *in vitro* (section 2.3.2). Three different treatments were used in this study;

1. Irradiated petiole explants cultured on non-irradiated MS medium supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA.
2. Irradiated petiole explants cultured on irradiated MS medium supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA.
3. Non-irradiated petiole explants cultured on irradiated MS medium supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA.

Radiated explants were exposed to gamma radiation dose and time stated in section 7.2.3. After irradiation, cultures were incubated in the dark overnight. All cultures were placed in the culture room at 25 ± 1 ^{o}C at 16 hours light and 8 hours dark for 8 weeks. The reason for experiments to be carried out on irradiated and non-irradiated MS medium was to compare the effects of irradiation on the culture medium in other words to see whether any effect on the chemical composition of the medium. Observation was done weekly and results were recorded.

7.2.5 Effect of Gamma Radiation on *In vitro* Propagated Shoots

Another experiment was conducted to study the effect of gamma radiation on regenerated shoots *in vitro*. Eight-week-old *in vitro* shoots were obtained from petiole explants cultured on MS medium supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA. Three different treatments were used in this study;

1. Irradiated *in vitro* shoots cultured on non-irradiated MS basal medium.
2. Irradiated *in vitro* shoots cultured on irradiated MS basal medium.
3. Non-irradiated *in vitro* shoots cultured on irradiated MS basal medium.

Cultures were maintained in the dark overnight right after irradiated. All cultures were maintained in the culture room at 25 ± 1 ^{o}C at 16 hours light and 8 hours dark for 8 weeks. Three-month-old plantlets were acclimatized and transferred to the green house. Growth and development of these plantlets were observed. Thirty replicates were used in each treatment.

7.2.6 Effect of Gamma Radiation on Callus Growth

Fresh callus (2.0g) obtained from leaf explants cultured on MS medium supplemented with 1.0 mg/l BAP and 2.0 mg/l 2, 4-D were exposed to gamma irradiation (0-60 Gy). Three different treatments were used in this study;

1. Irradiated callus cultured on non-irradiated MS medium supplemented with 1.0 mg/l BAP and 2.0 mg/l 2, 4-D.
2. Irradiated callus cultured on irradiated MS medium supplemented with 1.0 mg/l BAP and 2.0 mg/l 2, 4-D.
3. Non-irradiated callus cultured on irradiated MS medium supplemented with 1.0 mg/l BAP and 2.0 mg/l 2, 4-D.

Cultures were incubated in the dark overnight right after irradiated. All cultures were maintained in the culture room at 25 ± 1 ^{o}C at 16 hours light and 8 hours dark for 8 weeks. Growth and development of callus were observed. Thirty replicates were used in each treatment. At the end of 8-week culture period, total fresh weight of the callus was determined and morphological changes were observed and recorded.

Fresh weight percentage of irradiated callus was calculated according to the following formula:

$$\frac{\text{Total fresh weight of irradiated callus (8}^{th}\text{ week) (mg)} - \text{Total initial fresh weight of callus (mg)}}{\text{Total initial fresh weight of callus (mg)}} \times 100$$

7.2.7 Chlorophyll Extraction and Determination

Chlorophyll contents for irradiated callus were determined according to Arnon (1949). Irradiated callus was incubated for 7 days to study the effect of irradiation on

chlorophyll content in callus tissues. Two grams of callus was homogenized using a chilled mortar and pestle containing 10 ml of 80% acetone and some $MgCO_3$. Callus tissues were carefully ground while being kept chilled. The samples were extracted in a dark environment to prevent chlorophyll degradation. Once grinded tissue was fine and slurry, acetone was used to wash any sample material adhering to the pestle. Samples were centrifuged for 5 minutes at 200-300 g. The final extraction volume was made up to 50 ml with acetone. Readings of wavelength was taken at 645 nm and 663 nm using *Shimadzu s*pectrophotometer. Eighty percent acetone was used as the blank solution to zero the instrument.

7.2.7.1 Calculations of Total Chlorophyll Content In Irradiated Callus Tissues

The following calculation was made to ascertain sample chlorophyll concentrations. The chlorophyll concentrations were calculated as follows:

Chlorophyll a (mg/ml) = $12.7\ A_{663} - 2.69\ A_{645}$

Chlorophyll b (mg/ml) = $22.9\ A_{645-} 4.68\ A_{663}$

Where:

A_{645} = Absorbance at a wavelength of 645nm

A_{663} = Absorbance at a wavelength of 663 nm

Total chlorophyll (mg/ml) in original tissue sample = Chlorophyll a + Chlorophyll b.

Total chlorophyll (mg) in original tissue sample = Total chlorophyll (mg/ml) x Final volume (ml).

Total chlorophyll a (mg) in original tissue sample = Chlorophyll a (mg/ml) x Final volume (ml).

Total chlorophyll b (mg) in original tissue sample = Chlorophyll b (mg/ml) x Final volume (ml).

7.2.8 Soluble Protein Extraction

Soluble protein was extracted from irradiated callus using protein extraction buffer (Appendix 3) and centrifuged at 12 000 rpm at 4 ^{o}C for 20 minutes and the supernatant was used for soluble protein quantification.

7.2.8.1 Soluble Protein Analysis

Soluble protein concentration was determined by the Coomasie Blue Dye binding method of Bradford (1976) using bovine serum albumin (BSA) prepared in 0.15M NaCl as standard. The protein standard was prepared by adding 10-80 µg BSA in a volume up to 0.1 ml in test tubes. The volume in the test tube was adjusted to 0.1 ml with protein extraction buffer (Appendix 4), then 5.0 ml of protein reagent (Appendix 4) was added into the test tubes and the contents were mixed using vortex. The absorbance at 595 nm

was measured after 2 min and before 1 hour in a 3.0 ml cuvette against a reagent blank prepared from 0.1 ml of protein extraction buffer and 5.0 ml of protein reagent.

The weight of the protein was plotted against the corresponding absorbance to obtain a standard protein curve. Samples containing protein in volume of 50 µl was pipetted into test tubes. The volume in the test tubes was adjusted to 0.1 ml with protein extraction buffer and the protein content was measured following the procedure described above for protein standard curve.

7.2.9 Data Analysis

Data obtained were analyzed using Duncan's Multiple Range Test (DMRT). Mean with different letters in the same row differ significantly at p=0.05

7.3 RESULTS

Effects of gamma radiation on regeneration of shoots from petiole explants, regeneration of *in vitro* propagated shoots and callus growth of *G. jamesonii* Bolus ex. Hook f. were studied. Soluble protein content and chlorophyll content in irradiated callus of *G. jamesonii* were also investigated. Petiole explants, *in vitro* shoots and callus tissues were exposed to gamma radiation at different doses ranging from 10-60 Gray. The morphological changes and radio sensitivity effects on the radiated explants were observed.

7.3.1 Effects of Gamma Irradiation on Regeneration of Shoots from Petiole Explant

A significant decline was observed in the number of shoots regenerated from irradiated petiole explants as compared to the control, in all treatments. Numbers of shoots regenerated from irradiated petiole explants cultured on non-irradiated MS medium supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA was reduced to 6.6 ± 0.9 when explants were exposed to 20 Gray of irradiation dose (Table 7.1). Number of shoots regenerated was drastically reduced to 2.5 ± 4.6 (Table 7.1, Figure 7.1 c) when explants were exposed to 60 Gray of irradiation dose. Similar observation was obtained when irradiated explants were cultured on irradiated culture medium and non-irradiated explants cultured on irradiated medium. Number of shoots regenerated was reduced as the exposure to Gamma irradiation increased.

However, abnormalities in the shoots formed from irradiated explants were observed when explants were exposed to Gamma irradiation at 30 Gy (Figure 7.1 a), 40 Gy (Figure 7.1 b), 50 Gy and 60 Gy (Figure 7.1 c). All non-irradiated explants cultured on irradiated culture medium showed normal shoot formation. This showed that the irradiated medium did not influence shoot formation in the morphological changes of the shoots formed. It only influenced by the number of shoots formed due to the lack of medium quality. From the results, it was observed that irradiated explants cultured in non-irradiated medium showed positive morphological and irradiation response of explants in the formation of shoots.

Table 7.1: The effects of gamma irradiation on regeneration of shoots from petiole explants of *Gerbera jamesonii*. Irradiated explants were cultured on MS medium supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA. Cultures were incubated in the culture room at 25 ±1 ^{o}C with 16 hours light and 8 hours dark for 8 weeks.

Dose (Gy)	**Irradiated explant on non-irradiated medium (number of shoots) (mean ± SE)**	**Irradiated explant on irradiated medium (number of shoots) (mean ± SE)**	**Non-irradiated explant on irradiated medium (number of shoots) (mean ± SE)**
0 (control)	$7.5 \pm 0.4_{a}$	$7.5 \pm 0.4_{a}$	$7.5 \pm 0.4_{a}$
10	$7.0 \pm 1.1_{b}$	$6.0 \pm 1.7_{b}$	$6.3 \pm 1.7_{b}$
20	$6.6 \pm 0.9_{b}$	$5.4 \pm 2.0_{b}$	$6.0 \pm 2.4_{b}$
30	$5.3 \pm 1.5_{b,c}$*	$5.1 \pm 0.7_{b,c}$*	$5.4 \pm 0.9_{b,c}$
40	$5.0 \pm 0.8_{c}$*	$3.4 \pm 1.6_{d}$*	$4.8 \pm 0.5_{c}$
50	$3.8 \pm 2.3_{d}$*	$2.8 \pm 0.4_{d}$*	$3.4 \pm 2.6_{d}$
60	$2.5 \pm 4.6_{d}$*	$1.7 \pm 1.5_{d,e}$*	$2.1 \pm 1.5_{d}$

Mean ± SE, n=30. Mean with different letters in the same column differ significantly at p=0.05

* Formation of stunted and abnormal shoots

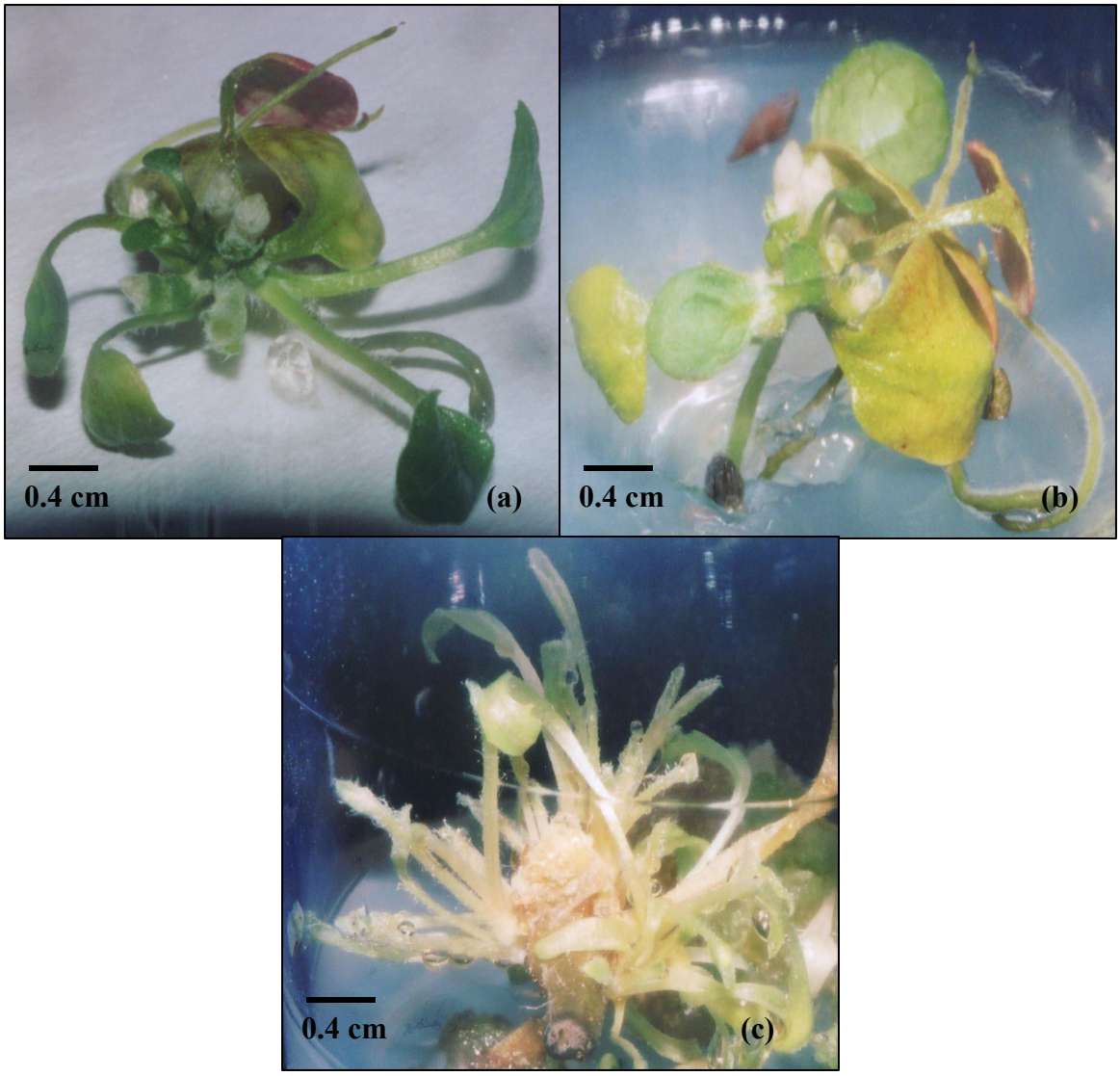


Figure 7.1: (a) Regeneration of shoots from irradiated petiole explants of *Gerbera jamesoni* at 30 Gy cultured on non-irradiated culture medium
(b) Regeneration of shoots from irradiated petiole explants of *Gerbera jamesoni* at 40 Gy cultured on non-irradiated culture medium
(c) Regeneration of shoots from irradiated petiole explants of *Gerbera jamesoni* at 60 Gy cultured on non-irradiated culture medium

7.3.2 Effects of Gamma Irradiation on Regeneration of *In vitro* Propagated Shoots

Similar observation was seen (as section 7.3.1) on effects of gamma irradiation on regeneration of *in vitro* propagated shoots. For all treatments, gradual decline in plant height was observed as the dose of gamma irradiation increased. Irradiated *in vitro* shoots cultured on non-irradiated medium showed effect as the morphological aspects of the plantlets showed irradiation response. As the gamma dose increased, plantlets showed irregular and abnormal characters. Compared to control treatment with 8.7 ± 0.6 cm, plantlet height was reduced to half with 4.1 ± 0.7 cm when irradiated *in vitro* shoots were cultured on non-irradiated medium and when plantlets were exposed to 60 Gy of irradiation dose (Table 7.2). Meanwhile, irradiated *in vitro* shoots cultured on irradiated medium showed drastic decline to 3.1 ± 2.0 cm when plantlets were exposed to 60 Gy of irradiation dose (Table 7.2). Non-irradiated *in vitro* shoots cultured on irradiated medium showed normal morphological growth when exposed to gamma irradiation. Three-month-old plantlets were acclimatized and transferred to the green house. Three-month-old plantlets obtained from irradiated *in vitro* shoots cultured on non-irradiated culture medium were acclimatized and transferred to the green house (Figure 7.2 a-d).

Table 7.2: The effects of gamma irradiation on regeneration of *in vitro* propagated shoots of *Gerbera jamesonii*. Irradiated shoots were cultured on MS medium supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA. Cultures were incubated in the culture room at 25 ±1 ^{o}C with 16 hours light and 8 hours dark for 8 weeks.

Dose (Gy)	**Irradiated *in vitro* shoots on non-irradiated medium [Plant height (cm)] (mean ± SE)**	**Irradiated *in vitro* shoots on irradiated medium [Plant height (cm)] (mean ± SE)**	**Non-irradiated *in vitro* on irradiated medium [Plant height (cm)] (mean ± SE)**
0 (control)	$8.7 \pm 0.6_{a}$	$8.7 \pm 0.6_{a}$	$8.7 \pm 0.6_{a}$
10	$6.8 \pm 1.1_{b}$	$6.0 \pm 0.2_{b}$	$8.2 \pm 1.5_{a}$
20	$5.7 \pm 0.9_{b}$	$5.3 \pm 1.4_{b,c}$	$8.0 \pm 0.8_{a}$
30	$5.4 \pm 1.2_{b,c}$	$4.7 \pm 0.7_{c}$	$7.4 \pm 2.2_{a,b}$
40	$4.9 \pm 0.5_{c}$*	$3.8 \pm 1.5_{c,d}$*	$7.1 \pm 0.5_{b}$
50	$4.6 \pm 1.5_{c}$*	$3.5 \pm 0.3_{c,d}$*	$6.8 \pm 1.3_{b,c}$
60	$4.1 \pm 0.7_{c}$*	$3.1 \pm 2.0_{d}$*	$6.5 \pm 0.6_{b,c}$

Mean ± SE, n=30. Mean with different letters in the same column differ significantly at p=0.05

* Formation of abnormal plantlets

Figure 7.2: (a) Three-month-old non-irradiated plantlets acclimatized and transferred to the green house
(b) Three-month-old irradiated plantlets at 10 Gy acclimatized and transferred to the green house
(c) Three-month-old irradiated plantlets at 20 Gy acclimatized and transferred to the green house
(d) Three-month-old irradiated plantlets at 30 Gy acclimatized and transferred to the green house

7.3.3 Effects of Gamma Irradiation on Callus Tissues

Generally, a significant decline in the fresh weight of irradiated callus cultured on non-irradiated medium compared to the control was observed. Growth responses of callus were strongly influenced by the radiation dose. The fresh weight of callus was reduced to 76.4 ± 2.2% compared to 89.7 ± 0.5% of control (Table 7.3) when callus tissues were exposed to 20 Gy Gamma irradiation (Figure 7.3 a). Total fresh weight of callus was further reduced to 64.3 ± 0.8% (Figure 7.3 b) and 59.4 ± 0.6% (Figure 7.3 c) when callus tissues were treated with 30 Gy and 40 Gy, respectively. With increasing dose of gamma irradiation, the colour of callus continued to darken and the tissues become browning and dissociation of the tissue was relatively poor in contrast to the control. At 60 Gy, the fresh weight of callus tissues was reduced by half compared to the control with only 40.2 ± 0.5% (Table 7.3).

Similar observation was noted from irradiated callus cultured on irradiated medium. As the gamma irradiation dose increased, proliferation of callus tissues declined and total fresh weight was reduced compared to control. No significant effect was observed when irradiated callus cultured on irradiated medium compared to irradiated callus cultured on non-irradiated medium. The fresh weight of non-irradiated callus cultured on irradiated medium was not significantly different from the control. The result showed that irradiated medium did not show much difference in the fresh weight of callus. The fresh weight yielded was almost similar to the control (Table 7.3). For

experiments regarding the total chlorophyll content and soluble protein content, only irradiated callus cultured on non-irradiated medium was used.

Table 7.3: The effects of gamma irradiation on callus of *Gerbera jamesonii*. Irradiated callus were cultured on MS medium supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA. Cultures were incubated in the culture room at 25 ±1 ^{o}C with 16 hours light and 8 hours dark for 8 weeks.

Dose (Gy)	**Irradiated callus on non-irradiated medium (Total Fresh Weight) (%) (mean ± SE)**	**Irradiated callus shoots on irradiated medium (Total Fresh Weight) (%) (mean ± SE)**	**Non-irradiated callus on irradiated medium (Total Fresh Weight) (%) (mean ± SE)**
0 (control)	$89.7 \pm 0.5_{a}$	$89.7 \pm 0.5_{a}$	$89.7 \pm 0.5_{a}$
10	$67.5 \pm 1.6_{b}$	$64.8 \pm 0.3_{b}$	$83.7 \pm 0.5_{a}$
20	$56.4 \pm 2.2_{c}$	$48.5 \pm 0.6_{c}$	$81.4 \pm 2.5_{a}$
30	$44.3 \pm 0.8_{c,d}$	$43.7 \pm 1.5_{c}$	$79.2 \pm 2.1_{a,b}$
40	$29.4 \pm 0.6_{e}$	$39.1 \pm 2.2_{c,d}$	$78.0 \pm 0.2_{a,b}$
50	$23.1 \pm 1.7_{e}$	$33.0 \pm 0.5_{d}$	$77.1 \pm 1.5_{a,b}$
60	$10.2 \pm 0.5_{e,f}$	$29.6 \pm 1.8_{d,e}$	$72.4 \pm 0.3_{b}$

Mean ± SE, n=30. Mean with different letters in the same column differ significantly at p=0.05

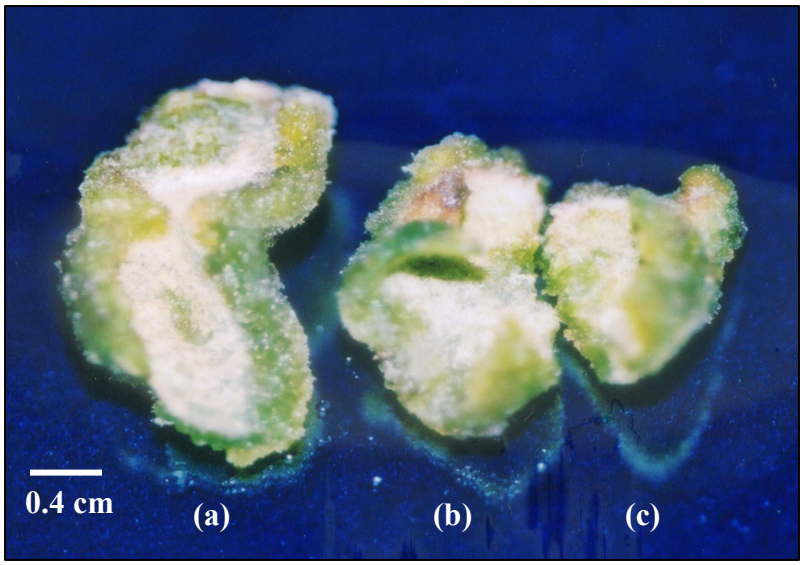


Figure 7.3: (a) Irradiated callus at 20 Gy cultured on non-irradiated culture medium
(b) Irradiated callus at 30 Gy cultured on non-irradiated culture medium
(c) Irradiated callus at 40 Gy cultured on non-irradiated culture medium

7.3.4 Chlorophyll a, Chlorophyll b and Total Chlorophyll Content of Irradiated Callus

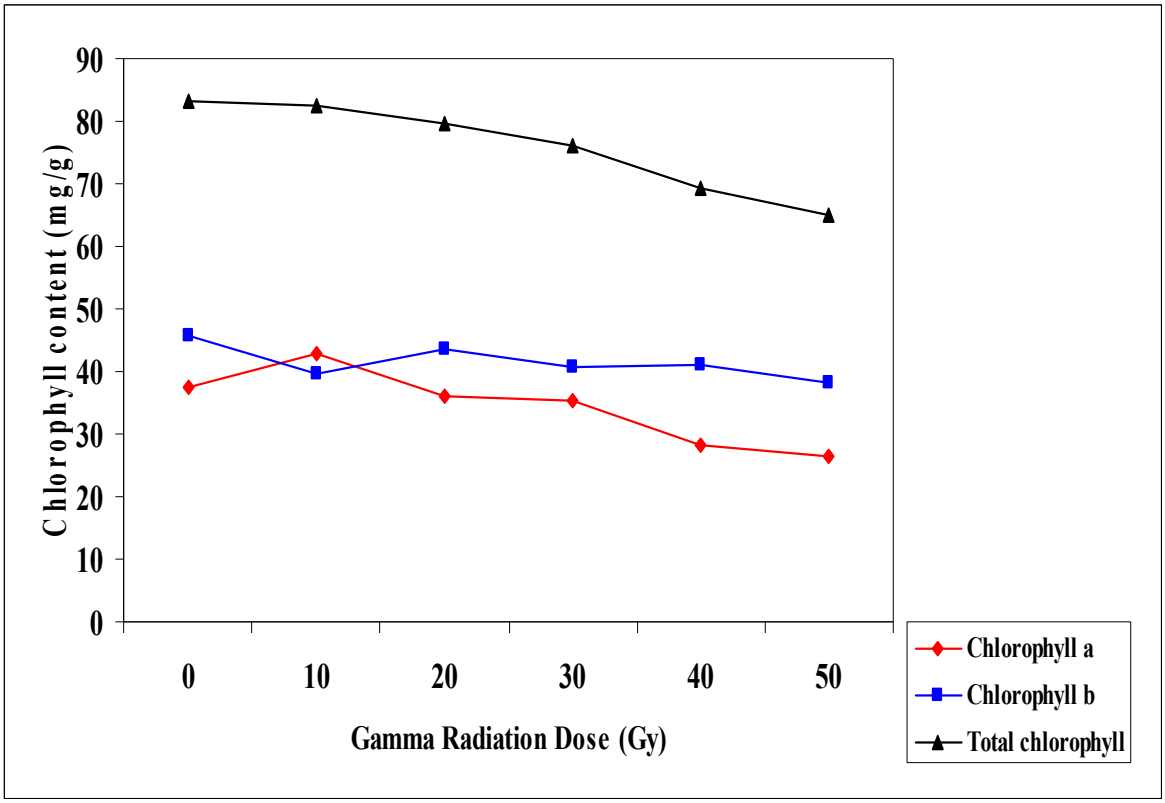
The chlorophyll content in fresh callus tissue is shown in Table 7.4. It was observed that as the irradiation dose increased, degradation of chlorophyll was expressed (Figure 7.4). The total chlorophyll content in the irradiated callus tissues decreased as the dose of irradiation increased. Total chlorophyll content in non-irradiated (control) was 83.2 mg/g and the amount of chlorophyll started to drop as the irradiation dose increased. Total chlorophyll was reduced to 79.8 mg/g when callus tissues were exposed to 20 Gy of Gamma irradiation. Total chlorophyll amount gradually reduced to 65.0 mg/g when callus tissue was exposed to 50 Gy. The experiment showed that gamma irradiation has significant effect in reducing the amount of chlorophyll in callus tissues.

Table 7.4: Chlorophyll a, chlorophyll b and total chlorophyll content of irradiated callus of *Gerbera jamesonii* at different doses of gamma irradiation

Gamma Radiation (Gy)	**Chlorophyll a (mg/g)**	**Chlorophyll b (mg/g)**	**Total Chlorophyll Content (mg/g)**
0 (control)	37.5 ± 1.1	45.7 ± 0.6	83.2_{a}
10	42.8 ± 2.4	39.8 ± 0.9	$82.6_{a,b}$
20	36.2 ± 2.6	43.6 ± 1.2	79.8_{b}
30	35.5 ± 0.8	40.7 ± 2.1	76.2_{b}
40	28.3 ± 1.7	41.0 ±0.6	69.3_{c}
50	26.3 ± 0.3	38.3 ± 1.7	$65.0_{c,d}$

Mean ± SE, n=30. Mean with different letters in the same column differ significantly at p=0.05

Figure 7.4: Chlorophyll a, chlorophyll b and total chlorophyll content of irradiated callus of *Gerbera jamesonii* incubated at 25 ± 1 ^{o} C with 16 hours light and 8 hours dark.



7.3.5 Soluble Protein Content in Irradiated Callus

Soluble protein content in fresh callus is shown in Table 7.5. Protein content from each treatment was obtained from protein standard curve plotted in Figure 7.5. The results showed soluble protein content in non-irradiated fresh callus was reduced during the 3rd day of treatment with 67.4 ± 0.8 µg/g (Table 7.5, Figure 7.6). However, soluble protein content started to increase during the 5th, 7th and 9th day of treatment. After the 9th day, soluble protein content was reduced and during day 15, soluble protein content in non-irradiated callus was 54.5 ± 2.5 µg/g (Table 7.5, Figure 7.6). It can be observed that, in the irradiated callus tissues, almost similar result was obtained where after the 9th day of exposure to Gamma irradiation, gradual reduction of soluble protein content was observed. Reduction of soluble protein content was observed in all treatments (exposure from 10 Gy to 60 Gy) as the incubation day increased to the 15th day of treatment. The results showed that soluble protein content decreased as the incubation day of non-irradiated and irradiated callus tissue increased.

Table 7.5: Soluble protein content (µg/g of fresh weight) in irradiated callus of *Gerbera jamesonii* at different doses of gamma irradiation

Days	Soluble Protein Content (µg/g of fresh weight)						
	0 Gy (control)	**10 Gy**	**20 Gy**	**30 Gy**	**40 Gy**	**50 Gy**	**60 Gy**
0	$74.7 \pm 1.2_{a}$	-	-	-	-	-	-
3	$67.4 \pm 0.8_{b,c}$	$69.8 \pm 1.5_{b}$	$67.7 \pm 0.6_{b,c}$	$66.5 \pm 1.2_{b,c}$	$64.0 \pm 0.3_{b,c}$	$62.5 \pm 0.7_{c}$	$61.6 \pm 1.2_{c}$
5	$69.2 \pm 1.7_{b}$	$68.7 \pm 1.3_{b}$	$69.9 \pm 0.8_{b}$	$68.3 \pm 1.2_{b}$	$67.1 \pm 0.4_{b,c}$	$64.1 \pm 1.5_{b,c}$	$62.1 \pm 0.7_{c}$
7	$69.6 \pm 2.2_{b}$	$66.2 \pm 0.7_{b,c}$	$67.0 \pm 0.8_{b,c}$	$67.6 \pm 1.1_{b,c}$	$68.1 \pm 0.5_{b}$	$66.5 \pm 0.3_{b,c}$	$65.0 \pm 1.2_{b,c}$
9	$69.9 \pm 1.4_{b}$	$65.2 \pm 0.9_{b,c}$	$67.8 \pm 0.9_{b,c}$	$66.5 \pm 1.5_{b,c}$	$67.1 \pm 2.1_{b,c}$	$66.4 \pm 1.8_{b,c}$	$65.7 \pm 1.6_{b,c}$
11	$63.0 \pm 2.2_{c}$	$62.9 \pm 0.4_{c}$	$63.7 \pm 0.2_{c}$	$62.8 \pm 0.7_{c}$	$61.5 \pm 2.2_{c}$	$61.3 \pm 0.6_{c}$	$60.8 \pm 2.5_{c}$
13	$59.72 \pm 1.8_{d}$	$60.2 \pm 3.2_{c}$	$61.1 \pm 2.8_{c}$	$60.3 \pm 1.8_{c}$	$59.6 \pm 0.7_{d}$	$58.3 \pm 0.9_{d}$	$57.0 \pm 1.9_{d}$
15	$54.5 \pm 2.5_{d}$	$55.0 \pm 1.7_{d}$	$55.5 \pm 1.6_{d}$	$54.8 \pm 0.9_{d}$	$54.1 \pm 1.3_{d}$	$53.6 \pm 2.4_{d}$	$52.7 \pm 1.4_{d}$

Mean ± SE, n=30. Mean with different letters in the same column differ significantly at p=0.05

Figure 7.5: Protein standard curve

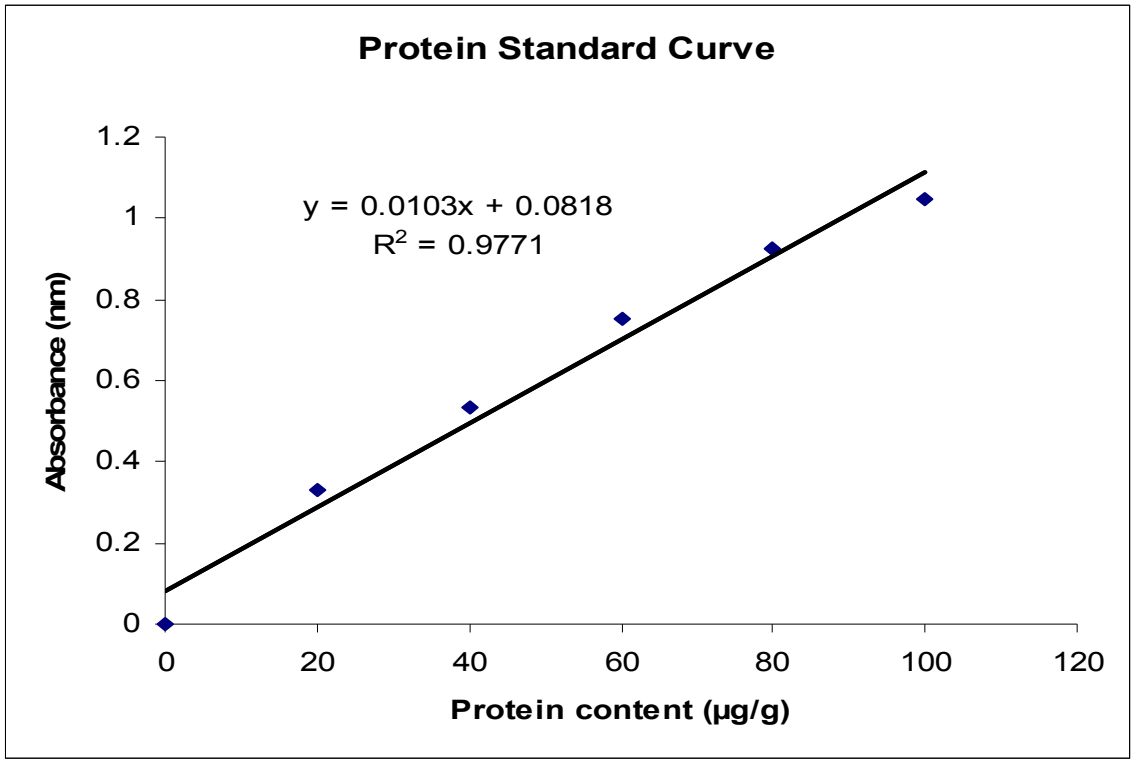
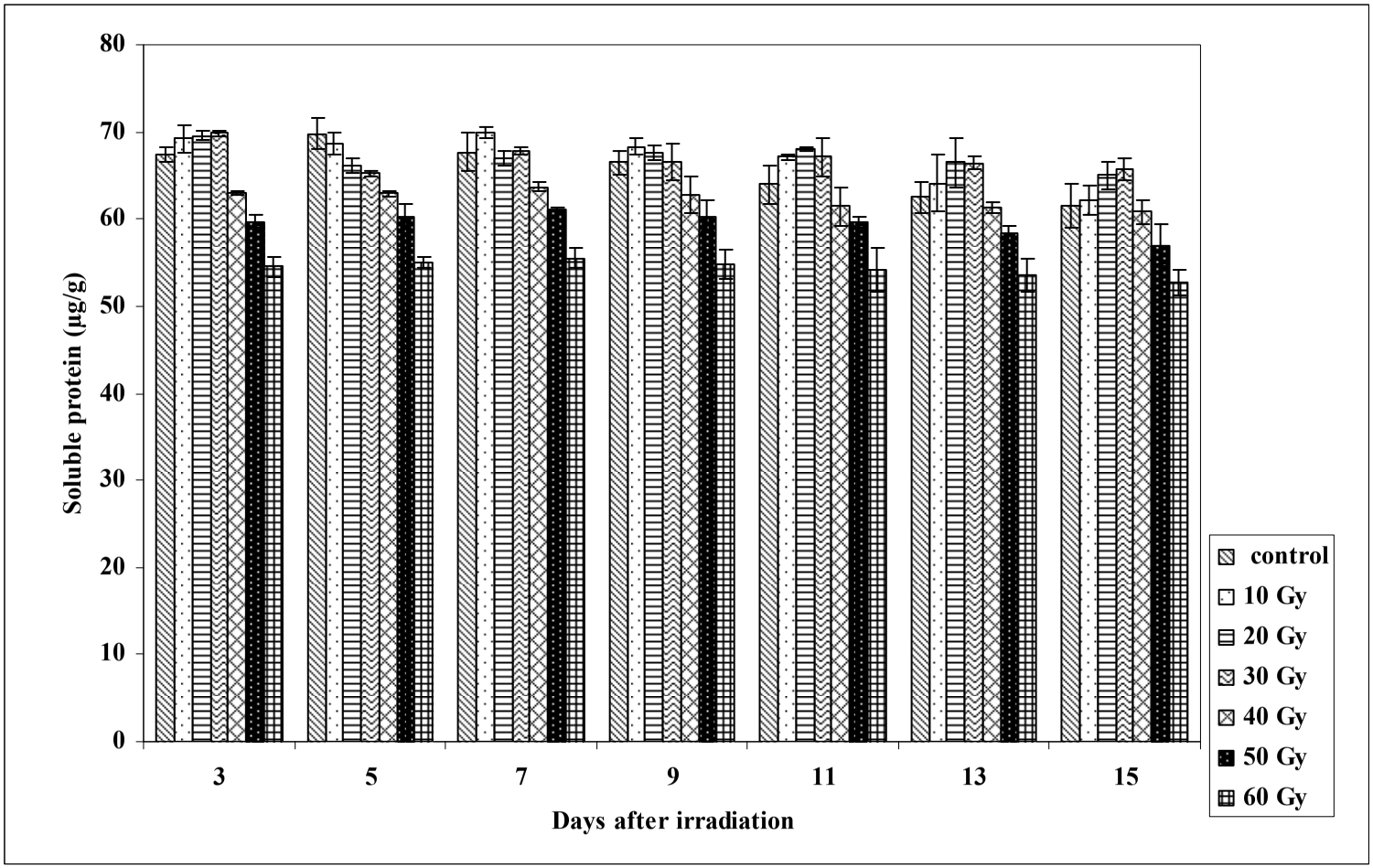


Figure 7.6: Soluble protein level (µg/g of fresh weight) in irradiated callus of *Gerbera jamesonii* incubated at 25 ± 1 ^{o}C and 16 hours light and 8 hours dark.



7.4 SUMMARY OF RESULTS

1) Irradiated explants, *in vitro* shoots and callus cultured on non-irradiated culture medium showed irradiation response

2) Significant decline in plant height was observed as the effect of gamma irradiation on regeneration of shoot from petiole explant as the dose of gamma irradiation increased up to 60 Gy.

3) Gamma irradiation also affected the growth of plantlet height as the *in vitro* regenerated shoots were exposed to gamma irradiation up to 60 Gy.

4) As the dose of gamma irradiation increased, inhibition of proliferation of callus tissues was observed.

5) Gradual decline was also observed in the total chlorophyll content as the dose of gamma radiation increased. Total chlorophyll content in irradiated callus at 10 Gy was 82.6 mg/g and the total chlorophyll content was reduced to 76.2 mg/g and 65.0 mg/g as the callus was irradiated at 30 Gy and 50 Gy, respectively.

6) The higher irradiation dose and the longer period of treatment, soluble protein content was found to be diminished.

7) Gamma irradiation has proven to affect in this case, decreased the amount of chlorophyll and soluble protein content in callus tissues.