CHEMICAL EVALUATION OF STRAWBERRY PLANTS AND FRUITS PRODUCED BY TISSUE CULTURING OF GAMMA IRRADIATED SEEDLINGS

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ABSTRACT

Studies were conducted to evaluate the influence of gamma irradiation preceding tissue culturing of strawberry seedlings (c.v. *Rosa linda*). The strawberry seedlings were irradiated using 8 doses of gamma rays i.e. 50, 75, 100, 125, 150, 250, 350 and 500 Gray. Tissue culture technique was applied on irradiated and un-irradiated (control) strawberry seedlings. Chemical characteristics of strawberry plant and fruit were studied as well as the early, total and exportable fruit yields. Data indicated that the radiation doses over than 250 Gray are lethal since the relatively high radiation doses i.e. 350 and 500 Gray affected markedly the strawberry seedlings and did not show any growth parameters. Carbohydrate fractions, total nitrogen, amino acids and protein contents showed remarkable increases in strawberry plant by the application of low doses (50, 75 and100 Gray) while they showed gradual decrease by increasing the radiation doses, whereas phenolic compounds content increased gradually by increasing radiation doses. Concerning strawberry fruits, the data revealed that the higher carbohydrate fractions content were obtained by the application of low doses (50, 75 and 100 Gray) and then the higher the radiation doses the lower the carbohydrate fractions content were obtained. On the other hand, amino acids, phenolic compounds and anthocyanin in fruits increased gradually by increasing the radiation doses. The fruit yields recorded remarkable increase. About 46% increase in the early yield was obtained after exposure to 50 Gray, while 51.9% increase in the total yield was obtained by the same dose. In addition, 56.4% increase in the total fruit yield was achieved after exposure to 75

Gray. Moreover, exposure to 50 Gray produced 27.7% increase in exportable fresh fruit yield at the end of the season.

It could be conclude that the exposure to low and safely doses of gamma radiation (50. 75 and 100 Gray) is recommended before tissue culturing of strawberry seedlings in order to achieve higher yield and high quality strawberry fruits either for local and/or foreign markets.

Key words: Gamma irradiation, tissue culture, strawberry, plantlets, exportable fruit yield.

INTRODUCTION

The use of ionizing radiation for keeping quality of foods and other biological application represents one of the important peace time applications of nuclear energy.

Agricultural scientists are usually looking into the possibility of making radiation a safe, successful and useful method in the biological applications, where the radiation process affects the physiological activity in the biological system. Therefore, radiation was used to generate genetic variation in plants. Irradiation with suitable doses of ionizing rays had been used as a stimulating agent for increasing yields (Beloshapkina, 1998) and/or improving quality of various crops (Kovacs and Keresztes, 2002).

Strawberry is considered one of the important horticultural crops for fresh consumption, food processing and exporting. Production of high quality strawberry cultivars is highly desirable for both consumer and exporter. Plant cell and tissue culture techniques play a significant role in plant improvement (Torres, 1989). Tissue culturing studies in strawberry conducted by Anderson *et al.* (1982) are of a special importance in case of asexually propagated plants. Couture and Willemot (1991) recorded that exposing strawberry to Gamma radiation of 300 or 2000 Gray and then storage at 4 °C for 32 days led to decreasing the rate of anthocyanin during storage. Maarouf (2001) reported that irradiated plantlets of strawberry with 55 Gray dose of gamma radiation increased the anthocyanin content. In contrast, El-Sharnoubi (2002) recorded that gamma irradiation reduced the pigments content in *Hypericum perforatum* grown in vitro. Arevals *et al.* (2002) found that gamma rays at doses of 100 and 150 Gray increased the phenolic compound contents in avocado fruit. Breitfellner *et al.* (2003) reported that exposing the strawberry to gamma rays (1000 up to 6000 Gray) had no effect on gallic acid, *p*-coumaric acid and caffeic acid, but there was an increase in 4-hydroxybenzoic acid. Abd El-Aziz (1998) stated that the percentage of reducing sugars decreased whereas, percentage of both non-reducing sugars and total soluble sugars increased with increasing gamma irradiation dose in cordyline leaves. Said (2001) reported that exposing peppermint plants to gamma rays at 10, 30 and 70 Gray increased total carbohydrate content. On the other hand, Maarouf (2001) found that irradiation of strawberry fruits with gamma rays had no remarkable effect in sugars content. Said (2001) stated that irradiation of peppermint plant led to gradual decrease in free amino acids content with increasing radiation doses (10, 30 and 70 Gray). On the other hand, the radiation dose 10 and 30 Gray caused a marked increase in soluble protein but the dose of 70 Gray decreased them in comparison with control. Farid *et al.* (1999) found that irradiation of sweet marjoram by low doses of gamma rays (10, 20 and 40 Gray) increased nitrogen content in the three harvested seasons. The highest nitrogen content was obtained at the radiation dose of 40 Gray. Concerning the yield, Maghraby (1997) stated that soybean seed yield significantly increased after exposure to gamma rays at doses 10 and 20 Gray in the first season, whereas the exposure to 40 Gray reduced significantly seed yield of soybean in the same season. Also, Beloshapkina (1998) reported that gamma irradiation treatment at 50 Gray significantly increased potential yields in the third vegetative generation of the strawberry cultivars *Senga sengana* and *Zhemchuzhnitsa* both in vitro and in vivo. On other hand, Hassanein *et al.* (1998) found that exposure to 40 Gray gamma radiation caused noticeable reduction in the yield of fababean seeds.

Few studies were carried out using tissue culturing technique preceding gamma irradiation treatment (Asad *et al.*, 1996 and Maarouf, 2001), whereas no study was carried out on gamma radiation treatment preceding tissue culture application in the filed of plant production.

Therefore, the present study is conducted to investigate the chemical evaluation of strawberry plants and fruits produced from tissue culture of gamma irradiated strawberry seedlings. The chemical characteristics of strawberry plant and fruit were studied. Moreover, the early, total and exportable fruit yields were taken into consideration also for their importance in the mass production.

MATERIAL AND METHODS

1. Sampling:

The seedlings of strawberry plants c.v. *Rosa linda* were obtained from the Strawberry and Non-Traditional Crops Improvement Center, Faculty of Agriculture, Ain Shams university. Where the experiments were carried out in the tissue culture laboratory of the strawberry and Non-Traditional Crops improvement Center, Faculty of Agriculture, Ain Shams university, Kalubeia and then the field trial was continued in Nobareia strawberry farm Behera, Egypt. The seedlings were cultivated in August (2003) in plastic pots 10cm contained Vem. : Peat : Sand by the ratio of 1:2:1 as recommended by Damiano (1977) and Badawi *et al.*, (1990). Pots were placed on benches under the condition of the greenhouse at 25°C± 2 with 53-68% humidity. The pots were irrigated twice weekly. Fertilization and macro-elements were applied every 10 days using 1g of compound fertilizer (19:19:19) per pot. After 45 days every plant developed two or three young runners, which became the materials of this investigation.

2. Radiation treatment:

The strawberry seedlings were divided into nine groups (45 seedlings) in polyethylene bags, eight of them were irradiated using 8 doses of Co_{60} gamma rays (50, 75, 100, 125, 150, 250, 350 and 500Gray) and the ninth group was used as control. Radiation treatments were carried out in the National Center of Radiation Research and Technology (NCRRT). The initial activity was 10400 CI [26/3/1988] Co_{60} and dose rate = 1.06608 K.Gray/Hour (Indian Research Unit, G.C.4000A). Atomic Energy Authorization, Nasr City, Cairo, Egypt.

3. Tissue culture and nursing:

Irradiated and un-irradiated strawberry runners were cut and washed thoroughly with tap water and then the leaves were removed. Under aseptic conditions, runners were sterilized using 70% ethanol (30 sec) followed by 25% Clorox (15 min) then rinsed several times using sterile distilled water. Under a binocular dissecting microscope the tissue removed from the end of the stem in lamina air flow cabinet. Shoot tips were excised at length of 1 mm, then cultured individually in culture tubes contained 15 ml of MS medium (Murashige and Skoog, 1962) + 1 mg/L BAP (Benzylaminopurine) + 1mg/L IBA (Indolbutyric acid) + 0.1 mg/L GA3 (Gibberellic acid).

Table 1: Composition of different strawberry culture media

Culture constituents	Meristem culture medium	Multiplication medium	Rooting medium
Macronutrients	Knop	Knop	Knop
Micronutrients	Knop	Knop	Knop
Vitamins mixture	MS	MS	MS
Hormonal substances (mg/L)	MS	MS	MS
BAP	1.0	0.5	---
IBA	1.0	1.0	1.0
GA_3	0.1	0.1	---
Glucose (g/L)	40.0	40.0	40.0
Agar (g/L)	5.2	5.2	5.2
Activated Charcoal (g/L)	___	___	0.5
*P*H	5.6	5.6	5.6

MS: Murashige and Skoog, BAP: Benzylaminopurine, IBA: Indolbutyric acid GA_3: Gibberellic acid

Cultures were maintained in the growth chamber at 25± 2°C under 16-h/day photoperiod (1000 lux) and 8 hours dark. After one month they were transferred to multiplication medium. Five weeks latter, they were transplanted to the third medium (rooting medium).

3.1. Acclimatization stage: Rooted plantlets were washed using current tap water, then disinfected by Benlate solution (1g/L) for 20 min. The plantlets were transferred to plastic pots (10 cm) containing a mixture of peat moss, vermiculite and sand (2:1:1), as recommended by Damiano (1977) and Badawi *et al.*, (1990). Pots were placed in a screen house for 4 weeks.

3.2. Nursery stage: The experimental design was the complete randomized blocks with three replicates.

3.3. Nursery soil preparations: Chicken Manure was added at the rate of 30 m^3/ Fed. Two weeks before cultivation, the soil fumigated with mixture of methyl bromide (98% methyl bromide and 2% chloropicrin) using 50 g/m^2. After two weeks, the transplants were spaced 1.5 ×1.5 meter in plots (25 m^2). Planting were at 21st of May as normal planting season. The sprinkler irrigation system was used at the spacing of 5m × 6m.

3.4. Fruiting stage: A complete randomized blocks design with three replicates was adopted. Each replicate consisted of all treatments.

Plants were arranged in four row beds, 120 cm apart. The plat area was 12 m^2. The beds were covered with 40 micron clear plastic mulch and the plants were covered with 80 micron plastic tunnels (70 cm height). The sprinkler irrigation was taken place in the first month after planting then the drip irrigation was applied after mulch until the end of the season.

3.5. Agricultural practices: Concerning cultivation, fertilization, irrigation, and pest–and disease–control were conducted as commonly followed for the commercial production of strawberry.

4. Chemical analysis:

4.1. Chemical composition of plants: Samples of ten plants from each treatment were randomly chosen after 60 days from transplanting and subjected to the following determining:

- **Total carbohydrates and its fractions** were extracted and determined according to A.O.A.C. (1990).
- **Free amino acids** were determined according to the method of Jayeraman (1985).
- **The protein content** was determined according to Kejldahl method A.O.A.C. (1990).
- **Total phenolic compounds** were determined according to Danial and George (1972).

4.2. Chemical composition of fruits: Samples of ten fruits from each experimental plot at the ¾ colored stage were randomly chosen at three different times of the season in beginning, mid and in the end of the season for the determination of chemical contents of strawberry fruits as follow:

- **Total sugars, reducing sugars, total carbohydrates, amino acids and phenolic compounds** were determined as mentioned above.
- **Anthocyanin** was determined according to De Loose (1970).
- **The early and total fruit yield** were determined as and the as weight of all harvested fruits (kg/100plant).
- **The exportable fresh fruit yield** was determined according to firmness, color, shape and size of fruits.

5. Statistical analysis:

Data were subjected to statistical analysis using the analysis of variance method. The means of treatments were compared using the least significant difference (L.S.D) at 0.05 level of probability according to Duncan (1955) Multiple Range test.

RESULTS AND DISCUSSION

The objective of this investigation is to evaluate the chemical composition of strawberry plants and fruits produced by tissue culturing of strawberry seedlings irradiated with gamma rays. Fruit yield and quality were also taken into consideration. The strawberry seedlings were irradiated using 8 doses of gamma rays ranged from low to high doses i.e. 50, 75, 100, 125, 150, 250, 350 and 500 Gray to cover a wide range of the application of gamma irradiation. The obtained results revealed that the relatively high radiation doses i.e. 350 and 500 Gray are lethal doses, since they affected strawberry seedlings markedly and did not show growth. Such effect of radiation on seedlings caused failure of tissue culturing technique in producing plantlets or any new small strawberry plant. Therefore, the survival plantlets were produced successfully only from the tissue culturing of 6 irradiated seedlings with the lower doses of radiation

1. Strawberry plants:

1.1. Total carbohydrate, total soluble and reducing sugars:

Data presented in Table (2) showed that the low doses of gamma radiation i.e. 50, 75 and 100 Gray in generally gave the highest content of total carbohydrates in both leaves and stems. Exposure to 100, 125 and 150 Gray are considered the optimum doses of radiation for giving the highest content of reducing sugars in leaves, while, the exposure to 125 and 150 Gray gave the highest content of total sugars in leaves and exposure to 50, 75 and 100 Gray were enough to gave the highest level of total sugars in stems. All the applied doses of radiation increased the total carbohydrate content in stems especially the exposure to 75 Gray increased significantly the total carbohydrates by 22% in stems in comparison with the untreated plants.

In addition, application of medium doses of 100, 125 and 150 Gray increased significantly the reducing sugars in leaves especially 125 Gray which recorded 28% increase. The highest increase (50%) of total sugars in leaves was achieved by 125 Gray which was preceded by a gradual increase started from 50 Gray dose of radiation. The same effect of the 125 Gray was observed in the case of reducing sugars in leaf since it gave the highest increase (28%). In this respect, our obtained results are in agreement with those observed by El-Shafey *et al.*, (1991) on *Sweet basil,* Abo El-Seoud *et al.*, (1991) on *Atropa belladonna* and Said (2001) on peppermint species.

The fore-mentioned data especially the results of the effect of 75 Gray on stems contents since it increased the reducing sugars content by ratio of 11.4% and total sugars by ratio of 15.8% and finally the total carbohydrates increased by ratio of 22%, led us to conclude that the performed irradiation treatments i.e. 75, 100 and 125 Gray stimulated the photosynthesis procedure either genetically or chemically which resulted in high synthesis of total sugars and reducing sugars and finally led to increasing in total carbohydrates content in strawberry leaves and stems.

Table 2: Carbohydrate Fractions of Strawberry Plants (*Rosa linda*) Produced From Tissue Culturing of Irradiated and Un-irradiated Strawberry Seedlings

Radiation doses (Gray)	Reducing sugar %		Total sugar %		Total carbohydrates %	
	Leaf	Stem	Leaf	Stem	Leaf	Stem
Control	2.264	4.283	3.145	5.753	9.075	10.540
50	2.407	4.442	3.387	6.612	10.670	10.930
75	2.431	4.772	3.465	6.664	10.020	12.860
100	2.852	5.318	3.685	6.550	9.581	12.530
125	2.900	4.485	4.734	5.946	8.828	11.070
150	2.683	4.249	4.370	5.676	8.570	10.650
250	1.435	4.047	2.624	5.367	8.155	10.610
L.S.D	0.3232	0.05626	1.037	0.4536	1.039	0.8055

1.2. Protein, amino acids and nitrogen contents:

Data recorded in Table (3) showed a gradual and significant increases in nitrogen content in stems with the 50, 75 and 100 Gray treatments, where it reached to the optimum increase (14%) by 75 Gray treatment. Reversely, no significant changes could be observed in nitrogen content in leaves except a significant decrease was occurred by the high dose of 250 Gray. Such results are supported by the study of El-Shafey *et al.*, (1991) on *Sweet basil.*

Data showed also a gradual increase in amino acids content in leaves by increasing the radiation doses till the significant increase which obtained by 100 Gray. In the case of stems, the 50 Gray was the optimum dose for increasing the amino acids content significantly followed by a gradual decrease by increasing the radiation doses but still around the untreated (control) value. The same trend was observed in case of protein content since the 50, 75 and 100 Gray were the optimum doses for increasing the protein content significantly in both leaves and stems in general.

The data concerning the amino acids and the protein content in both leaves and stems are concomitant to each other under the influence of the different doses of radiations. That means the gamma rays stimulate the synthesis of the amino acids which consequently resulted in increasing in protein content in the plant either genetically or chemically. The high content of amino acids leads to high content of protein. Similar observations were reported by Sakr (1992) on protein content in leaves, stems and flowers of *Hyoscyamus muticus L* due to the accumulation of protein biosynthesis.

Table 3: Protein, Free Amino Acids and Nitrogen Contents of Strawberry Plants (*Rosa linda*) Produced From Tissue Culturing of Irradiated and Un-irradiated Strawberry Seedlings

Radiation doses (Gray)	Amino acids%		N %		Protein %	
	Leaf	Stem	Leaf	Stem	Leaf	Stem
Control	1.90	3.68	4.32	1.70	25.16	10.59
50	1.97	4.76	4.37	1.90	25.85	11.88
75	2.10	4.25	4.44	1.941	26.63	12.13
100	2.34	3.97	4.20	1.70	27.10	10.59
125	2.31	3.65	4.18	1.69	26.13	10.55
150	1.81	3.54	3.98	1.53	24.85	9.54
250	1.67	3.34	3.53	1.46	22.06	9.14
L.S.D	0.3898	0.3513	0.5709	0.05626	1.165	0.4172

The increases of the nitrogen content by ratio of 14.1% and the amino acids content by ratio of 15.4% concomitant with 14.5% increase in protein content in stems under the exposure to the same dose of 75 Gray, led one to gave an explanation that the radiation might be enhance the biosynthesis of the amino acids based on the increase of the nitrogen uptake in plants which finally led to the increase in protein biosynthesis.

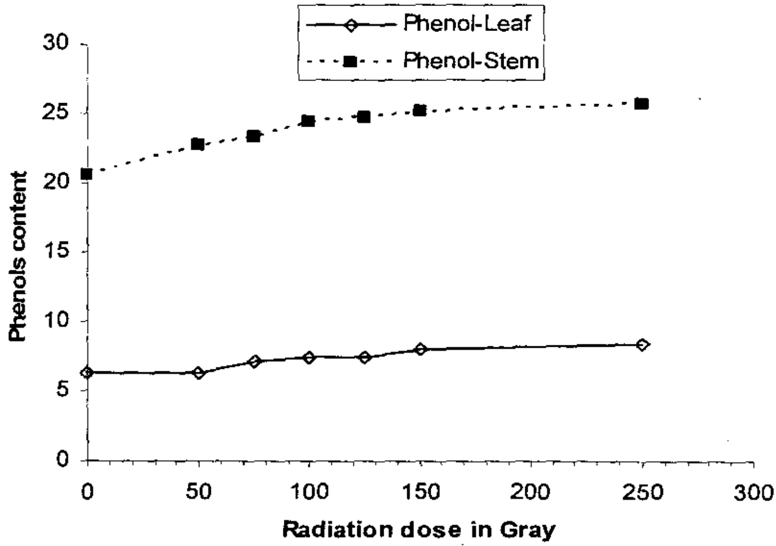

Figure (1): Phenols content (g/100g dw) of strawberry leaves and stems produced from tissue culturing of irradiated and un-irradiated strawberry seedlings

1.3. Phenolic compounds:

The phenolic compounds content are illustrated graphically in Figure (1). The obtained data indicated that the phenols content of strawberry plants increased gradually by increasing irradiation doses in both leaves and stems.

Concerning the observed data on phenolic compounds content, it could be concluded that the changes in phenolic compounds content are attributed to the effect of gamma radiation on the phenolic biosynthesis, which depends on the activities of the oxidative enzymes and its relation to the browning process. Such increase in the phenols content was in a harmony with the increase in the activities of the oxidative enzymes which could be observed through the browning of the plants, Juangbhanich (1971) on banana and El-Shemy *et al.*, (1990) on grapefruits.

2. Strawberry fruits:

Regarding the fruits yielded from the tissue culturing of irradiated and un-irradiated strawberry seedlings, the strawberry fruits as the end product of the strawberry plants were chemically evaluated by following up the desired chemical constituents of the fruits such as total carbohydrates, total soluble sugars, reducing sugars, free amino acids, phenolic compounds and anthocyanin contents along the season of fruit production, at the begging, at the mid and at the end of the season. In addition, the strawberry fruit yield was determined including total yield and exportable fresh fruit yield to evaluate the total yield quantitatively and qualitatively.

2.1. Carbohydrate fractions

One of the favorite chemical characteristic of strawberry fruits is the level of total carbohydrate and its fractions i.e. total soluble and reducing sugars content. They were determined as g/100g DW. of strawberry fruits.

2.1.1. Reducing sugars content:

Data illustrated in Figure (2) showed that the irradiation doses of 150 and 250 Gray gave the highest content of reducing sugars at the beginning of the season, however 75 and 100 Gray gave the highest content in both end and mid of the season. In general, the 100 Gray recorded the highest content of reducing sugars at the mid of the season.

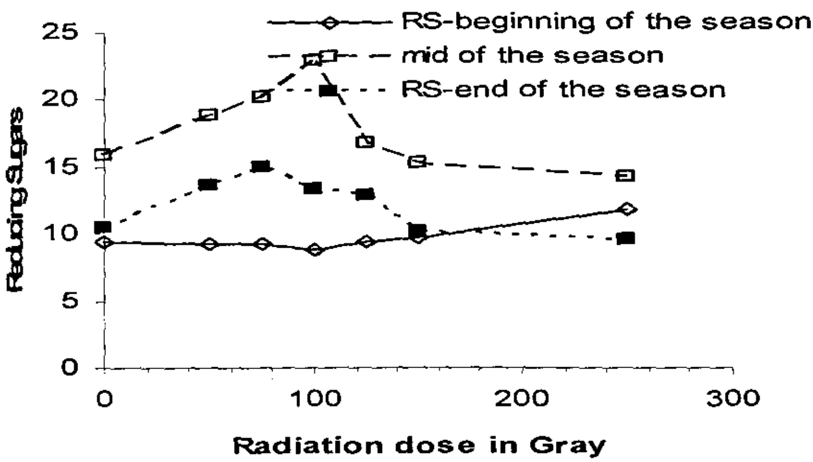

Figure (2): Reducing sugars of strawberry fruits (g/100g DW) produced from tissue culturing of irradiated and un-irradiated strawberry seedlings

2.1.2. Total soluble sugars content:

Data shown in Figure (3) showed that at the beginning of the season, 50 and 75 Gray treatments gave highest content of total soluble sugars (21.80 and 22.45 g/100g DW) respectively, followed by a gradual decrease till the dose of 250 Gray which gave the lowest content of total sugars (17.64 g/100g DW) in comparison with control (20.64 g/100g DW). The same trend of effect of the same doses could be observed at the end of the season, the 50 and 75 Gray treatments gave the highest value of total soluble sugars (22.72 and 23.41 g/100g DW) respectively. While at the mid of the season, 75 and 100 Gray treatments gave the highest content of total soluble sugars (26.27 and 28.57 g/100g DW) respectively.

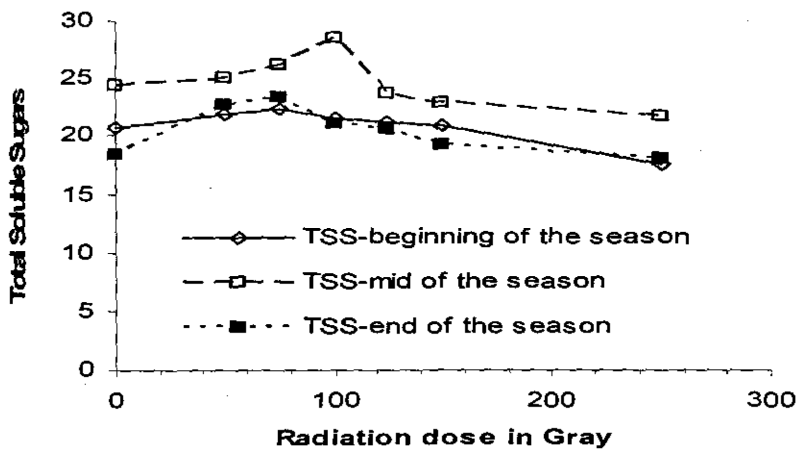

Figure (3): Total soluble sugars of strawberry fruits (g/100g DW) produced from tissue culturing of irradiated and un-irradiated strawberry seedlings

2.1.3. Total carbohydrates content:

Illustration of data in Figure (4) represented the total carbohydrates content in strawberry fruit showed that 50 Gray treatment increased the total carbohydrate content (26.47, 33.22 and 31.00 g/100g DW) along the season; at the beginning, mid and at the end of the season respectively. A gradual decrease in total carbohydrate was occurred by increasing radiation doses till the 250 Gray treatment (20.57, 24.61 and 24.56 g/100g DW) at the fore-mentioned three parts of the season respectively in comparison with the control values 25.61, 30.60 and 27.62 g/100g DW respectively.

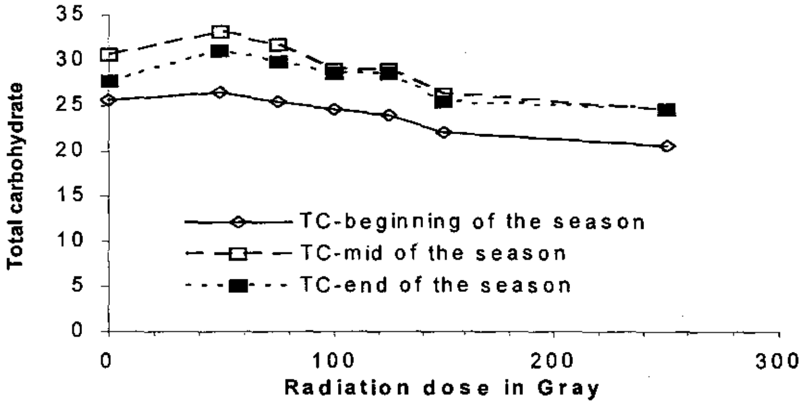

Figure (4): Total Carbohydrate of Strawberry Fruits (g/100g DW) Produced from Tissue Culturing of Irradiated and Un-irradiated Strawberry Seedlings

These results are in agreement with those mentioned by Sakr (1992) on *Hyoscyamus muticus L.*, Hassanein *et al.*, (1998) on faba bean seeds and Said (2001) on peppermint plant.

Concerning the effect of gamma irradiation followed by tissue culturing of strawberry seedlings on the total carbohydrates and its fractions; reducing sugars and total soluble sugars content in strawberry fruits, the same shape of pyramidal effect could be observed along the three periods of the season for the contents of total carbohydrates and its fractions. The highest pyramidal shape observed at the mid of the season and the lowest pyramidal shape obtained at the end of the season, while, the medium pyramidal shape found at the end of the season. The tops of all pyramidal shapes of effect of gamma rays on the total carbohydrates and its fractions almost restricted between 50 and 100 Gray doses of radiation. These abovementioned results led one to conclude that the low doses of gamma rays enhance the biosynthesis of the carbohydrate fractions either genetically or chemically at the begging of the mature stage of the fruiting (at the begging of the season which always gave the lowest pyramidal shape of contents). By the developing of the mature stage of fruiting, the biosynthesis of the carbohydrate fractions increased and reached to their highest values at the mid of the season and then started to slow down by the ending of the season which may be attributed to the increasing in the normal catabolism process in the plant.

2.2. Free amino acids content:

The graphically illustrated data in Figure (5) showed the free amino acids in strawberry fruits (g/100g DW) in response to gamma irradiation followed by tissue culturing along the season. The obtained data revealed that the increasing in gamma irradiation doses led to the increasing in free amino acids content gradually till reached its maximum values i.e. 4.47, 6.10 and 8.837 g/100g DW at the beginning, mid and at the end of the season respectively by the highest applied 250 Gray dose of radiation in comparison with the control values (3.65, 3.84 and 7.107 g/100g DW. respectively). The dramatic increase started at 150 Gray dose of radiation and then followed to 250 Gray. This type of effect was clear along the different times of the season, since at the beginning of the season 22% increase in the free amino acids content was detected, 58% increase was determined at the mid of the season and by the end of the season 24% increase was observed. Several investigations concerning the effect of gamma irradiation on protein and free amino acids contents are in agreement with our obtained data such as those observed by El-Ghinbihi (1980) and Abo El-Seoud *et al.*, (1991)].

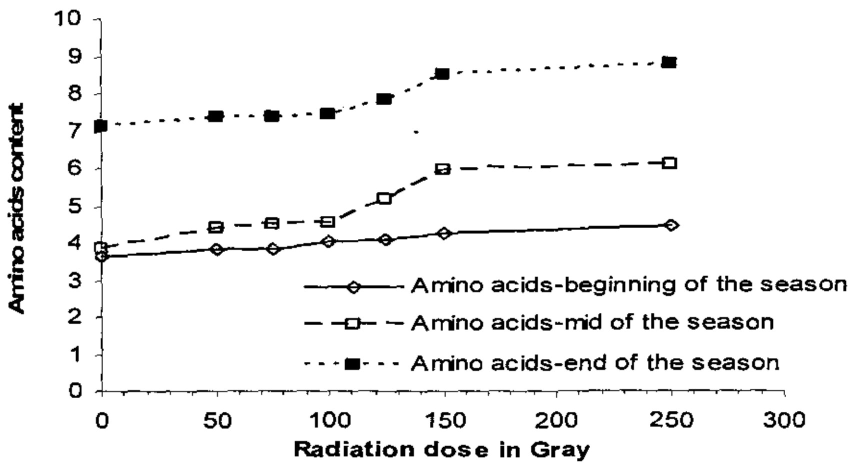

Figure (5): Free amino acids content of strawberry fruits (g/100g DW) produced from tissue culturing of irradiated and un-irradiated strawberry seedlings

The increase in the free amino acids content in strawberry fruits could be results of the increase in the nitrogen content and then the increase in the free amino acids content in the plants especially in the stems which suggested before in discussing the increasing of the nitrogen and amino acids contents in the strawberry plants.

2.3. Phenolic compounds content:

The phenolic compounds content are illustrated in Figure (6). The data indicated that all irradiation doses caused slight gradual increase in total phenolic compounds content with increasing radiation doses, and reached to the highest content (2.12, 3.0 and 2.68 g/100g DW) by applying the 250 Gray dose of radiation at the beginning, mid and at the end of the season respectively. Whereas, the phenolic compounds content in the un-irradiated treatment at the same periods were 1.53, 2.00 and 2.16 g/100g DW respectively.

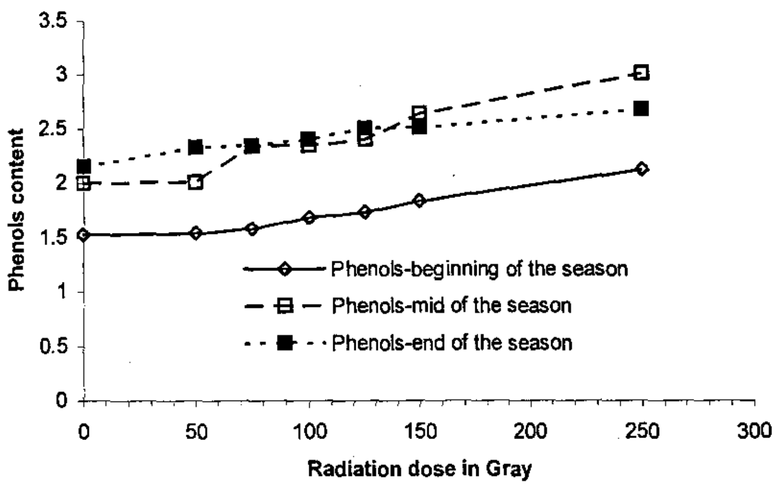

Figure (6): Phenols content of strawberry fruits (g/100g DW) produced from tissue culturing of irradiated and un-irradiated strawberry seedlings

The increase in phenolic compounds was more pronounced at the mid of the season. Such change in the phenolic compounds in irradiated plants may be attributed to the effect of gamma radiation on the phenolic biosynthesis and the related oxidative enzymes, which could be observed as a result of increasing the browning of the fruits. The increase in phenol compounds are attributed to the increase in L-phenilalanine ammonialyase activity by the exposure to 150 Gray in fresh Hass avocados (Lourdes *et al.*, 2002).

2.4. Anthocyanine pigment content

The data graphically illustrated in Figure (7) showed the anthocyanine content (g/100g FW) in strawberry fruit in response to gamma irradiation treatments followed by tissue culturing of strawberry seedlings. The obtained data revealed that irradiation doses caused a gradual increase in the content of anthocyanine with increasing radiation dose along the season, at the beginning, mid and at the end of the season. The highest applied radiation dose of 250 Gray gave the highest content of anthocyanine at all periods (78.00, 86.29 and 70.50 g/100g FW. respectively) in comparison with control (60.72, 65.25 and 48.16 g/100g DW. respectively).

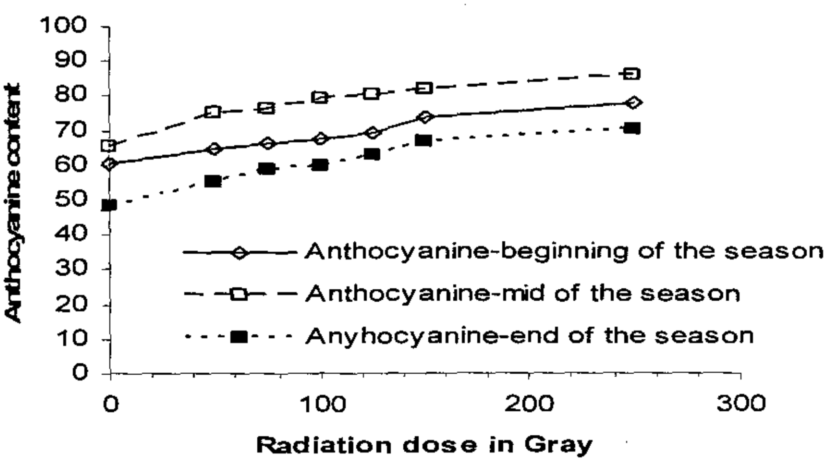

Figure (7): Anthocyanine content of strawberry fruits (%) produced from tissue culturing of irradiated and un-irradiated strawberry seedlings

Concerning the effect of radiation on the anthocyanine content in strawberry fruits, the results showed gradual increase at all different periods of the season and scored the maximum increase 46% at the end of the season by the highest dose of 250 Gray of radiation preceded by 32% increasing at the middle and 28% increasing at the beginning of the season. These results are in agreement with the finding of Youssef *et al.*, (1998) on *Melaleuca armillaris* and Maarouf (2001) on strawberry. The increase of the anthocyanine content in strawberry fruits by exposure to gamma radiation is deciphered by the action of radiation for developing more anthocyanine via two ways of actions, first by increasing the amount of pigmentation (anthocyanine) in pigmented cells and second by increasing the number of pigmented cells in the cell suspension culture (Sato *et al.*, 1996).

3. Strawberry fruit yield:

For more detection and evaluation of the effect of gamma irradiation on strawberry fruit yield, two types of yield were determined, the early and the total fruit yield as follow:

3.1. Early fruit yield:

It is clear from the obtained data (Table 4) that there was significant increase in the early yield of strawberry fruits by the application of low doses i.e. 50, 75, 100 and 125 Gray of gamma radiation, since they gave the highest earlier yield of the fruits (5.633, 5.313, 4.097 and 4.097 Kg/100 plants respectively). The highest dose of 250 Gray of gamma radiation gave the lowest content of early yield (2.217 Kg/100 plant) in comparison with 3.853 Kg/100 plant yielded by the control treatment.

Generally, and based on the obtained data one could conclude that the 50 Gray dose of radiation is the optimum dose for yielding the highest fruit yield of strawberry (46% increase in fruit yield). The increase of the radiation doses decreased the early fruit yield gradually till the dose of 150 Gray, which gave the same average as un-irradiated plants.

3.2. Total fruit yield:

Data in Table (4) showed total fruit yield (Kg/100 plant) in response to gamma irradiation treatments followed by tissue culturing of strawberry seedlings. The results indicated that the low doses of gamma radiation i.e. 50, 75 and 100 Gray significantly increased the total fruit yield and reached to 16.48, 16.97 and 14.82 (Kg/100 plant respectively), while the un-irradiated plants gave 10.85 (Kg/100 plants). On the other hand, there was a significant decrease in the total fruit yield (lower than un-irradiated plants) under the influence of the high radiation doses i.e. 150 and 250 Gray which yielded 9.67 and 7.58 (Kg/100 plant respectively).

Table 4: The early and the total yields of strawberry fruits (*Rosa linda*) produced from tissue culturing of irradiated and un-irradiated strawberry seedlings

Radiation dose (Gray)	Early yield (Kg/100 plant)	Total yield (Kg/100 plant)
Control	3.853	10.850
50	5.633	16.480
75	5.313	16.970
100	4.097	14.820
125	4.097	11.543
150	3.620	9.670
250	2.217	7.580

Regarding the obtained data on the yield of strawberry fruits either the early yield or the total yield and based on our findings, we could emphasize that the low doses of radiation either 50 or 75 Gray are the optimum doses for achieving the highest fruit yield of strawberry as early or as total yield. Increasing by a ratio of 46% in the early yield of strawberry fruit could be obtained by applying the radiation dose of 50 Gray, while, 51.9% increase in the total yield could be obtained by applying the same dose. Moreover, 56.4% increase in the total fruit yield of strawberry was obtained by applying the 75 Gray. Our obtained data are in agreement with those recorded by Grossman and Craig (1983) on progenies from *Pelargonium hortorum* plant, Mazrou (1993) on *Salvia officinalis*, Zheljazkov *et al.*, (1996) on *Mentha arvensis* cultivar Mentolana, Said (2001) on peppermint and Maarouf (2001) on strawberry.

The high yields resulted by the low doses of gamma irradiation could be explained by the stimulation in the growth of the plants by increasing the naturally occurring of auxin hormone in the plant via increasing in auxin biosynthesis. While the decrease in the yields by applying the high doses of 150 and 250 Gray may be attributed to the occurrence of growth inhibitors which decrease auxin content via decreasing auxin biosynthesis. Such conclusion is in a harmony with those recorded by Vackova and Kutacek (1976).

4. Exportable fresh fruit tield:

Data presented in Table (5) showed that at the beginning of the season 50, 75, and 100 Gray doses of radiation gave the highest values (80.51, 78.62 and 71.73% respectively), of exportable fresh fruit yield compared with the control (68.35%), while 150 and 250 Gray treatments gave the lowest value (61.30 and 50.41% respectively).

Table 5: The exportable fresh fruit yield of strawberry fruits (*Rosa linda*) produced from tissue culturing of irradiated and un-irradiated strawberry seedlings

Radiation dose (Gray	Exportable fresh fruit yield %		
	A	B	C
Control	68.35	71.66	58.81
50	80.51	84.22	74.93
75	78.62	82.63	70.13
100	71.73	75.43	63.59
125	67.90	71.59	57.17
150	61.30	65.10	46.08
250	50.41	51.79	27.63

Where: A = at the beginning of the season, B = at the mid of the season, C = at the end of the season.

Also, at the mid of the season 50, 75 and 100 Gray gave the highest value (84.22, 82.63 and 75.43% respectively), compared with the control (71.66%), while the high doses (150 and 250 Gray) gave 65.10 and 51.79%, respectively. Homeopathic effect could be observed by the same doses of radiation at the end of the season (74.93, 70.13 and 63.59% respectively), compared with the control (58.81%).

The above-mentioned data showed a significant increase in the exportable fresh fruit yield by the applied doses of 50 and 75 Gray of gamma radiation followed by tissue culture technique along the season of the fruiting. Gradual receding of exportable fresh fruit percentages were detected by increasing gamma radiation doses over than 50 Gray till 125 Gray which gave the same average as given by control.

CONCLUSION

Concerning the effect of gamma irradiation treatments preceding tissue culture application on the exportable fresh fruit yield of strawberry which represent one of the two main objectives of strawberry production, we could state that the low dose of 50 Gray of gamma radiation is an optimum dose of radiation for achieving this goal. About 18% increase in the exportable fresh fruit yield of strawberry was scored at the beginning and at the mid of the season of fruit production. In this respect, the most important effect was the increasing of this yield by a ratio of 27.7% of exportable fresh fruit by the end of the season under the influence of the same dose i.e. 50 Gray of gamma radiation.

In general, the increase in fruit yield of strawberry produced from tissue culturing of irradiated seedlings by the relatively low doses i.e. 50, 75 and 100 Gray could be explained by the stimulating effect of low radiation doses on strawberry seedling which they increased the metabolites in the plant either genetically and/or chemically which represents the growth components i.e. carbohydrate fractions (22% increase in total carbohydrate, 15.8% increase in total soluble sugars and 11.4% increase in reducing sugars in plant) and free amino acids (14.1% increasing), in addition to the increase in other growth elements such as phenols and pigment. All pre-mentioned increases together led to high formation of the end products of the strawberry plant i.e. the strawberry fruit.

Generally from both economic and biochemical point of few, it is quite clear that irradiation of strawberry seedlings using the relatively low and safely radiation doses i.e. 50, 75 and 100 Gray help the tissue culturing of strawberry seedlings successfully in producing good plants which lead to high yield with high quality of strawberry fruits, either in local market and/or for foreign markets (exportable fresh fruit yield). So it could be recommended the low doses of 50 and 100 Gray of gamma radiation as the optimum doses followed by tissue culturing of strawberry seedlings for achieving these goals.

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التقييم الكيميائى لنباتات وثمار الفراولة الناتجة من زراعة الأنسجة لشتلات معاملة بأشعةجاما

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أجريت هذه الدراسة لتقييم أستخدام أشعة جاما قبل تنمية شتلات الفراولة بإستخدام تقنية زراعةالأنسجة (صنف روزالندا). تم تشعيع شتلات الفراولة (المدادات) بثمانية جرعات من أشعة جاما هى 50 و 75 و 100 و 125 و 150 و 250 و 350 و 500 جراى. وقد تم تطبيق تكنيك زراعة الأنسجة على شتلات الفراولة المعاملة بالأشعاع والغير معاملة بالأشعاع وقد تم دراسة الخصائص الكيميائية لنباتات وثمار الفراولة بالأضافة الى محصول الثمار الكلى والقابل للتصدير. أشارت النتائج المتحصل عليها الى الأتى:

- جرعات الأشعاع الأعلى من 250 جراى كانت جرعات مميتة مثل 350- 500 جراى حيث أنهم أدوا الى فشل زراعة الأنسجة فى أنتاج النبيتات أو أى نبات صغير جديد.
- الجرعات المنخفضة من الأشعاع 50 - 100 جراى أدت الى زيادة الكربوهيدرات بأنواعها والنيتروجين الكلى والأحماض الأمينية والبروتينات فى نباتات الفراولة ثم حدث نقص تدريجى بزيادة الجرعة الأشعاعية بينما زاد محتوى المركبات الفينولية تدريجيا بزيادة الجرعات الأشعاعية.
- وبالنسبة لثمار الفراولة أشارت النتائج الى حدوث زيادة فى محتوى الكربوهيدرات بأنواعها بأستخدام الجرعات المنخفضة من الأشعاع 50 - 100 جراى يتبعها نقص بزيادة الجرعات الأشعاعية ومن ناحية أخرى فأن المركبات الفينولية والأنثو سيانين زادت تدريجيا بزيادة الأشعاع.
- أوضحت النتائج المتحصل عليها بأن هناك 51.9-59.4 % زيادة فى محصول الثمار الكلى بأستخدام الجرعات 50-75 جراى على التوالى علاوة على ذلك فأن الجرعة 50 جراى أدت الى 27.7 % زيادة فى محصول الثمار القابلة للتصدير فى نهاية الموسم.

من النتائج المتحصل عليها من البحث يمكن التأكيد على أن الجرعات المنخفضة من الأشعاع 50 75 و100 جراى يمكن أستخدامها قبل زراعة الأنسجة الخاصة بإنتج الفراولة وذلك للحصول عل محصول عالى جدا من الفراولة بزيادة تصل الى 50 % من المحصول الكلى بالأضافة الى إنتاج ثمار عالية الجودة سواء للأسواق المحلية أو الخارجية وجدير بالذكر أن الجرعات المستخدمة من الإشعاع والتى أعطت نتائج جيدة هى الجرعات المنخفضة والتى تعتبرمن الجرعات الأكثر أمانا فى الأستخدام ولايعتبر أستخدامها من الناحية التطبيقية مكلف إقتصاديا بالمقارنة بإنتاجيتها العالية والتى تصل الى 50 % زيادة فى إنتاج المحصول عالى الجودة وزيادة القيمة الغذائية لها بزيادة محتوى العناصر الغذائية مثل الكربوهيدرات والبروتين بالإضافة الى جودة الشكل واللون.

قام بتحكيم هذا البحث: أ.د/ نجاح الشحات، أ.د/ زينب أحمد عبده