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Effect of Re-irradiation on Vegetative Traits of Apple *Malus domestica In Vitro*

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Abstract

Plantlets of Apple Royal Gala were irradiated twice for Gamma rays at different doses 0, 10, 20, 25, 30, 40 Gy. Stem cuttings (with two nodes) and the leaf segments were excised, as well as the leaf segments were directly irradiate for once with the same doses. The cultures were incubated in the growth room chamber at 25°C±2 under 16 h light and 8 h dark. Data on percentage of survival %, Shoots per explant and plant height were taken. According to the percentage of survival criteria, Stem cuttings showed a high percentage (100%) for one exposing at all doses Whilst that twice exposing showed a decrease in survival reached 10 % at 30 Gy with completely failed to grow at 40 Gy. Leaf segments also showed gradually decreased in survivals at increasing doses for one and twice exposing. The irradiated leaf segments were directly exposed affected at all doses of radiation, the lowest survival was 6% at 40 Gy. The results also showed the negative effect of irradiation which was repeated twice on the number of shoots and plant height for stem cutting and leaf segments. It is clear that leaf segments which directly irradiated are affected by the reduced formation of the vegetative shoots by increasing of radiation doses.

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Introduction

Plant Tissue Culture Technique was characterized as one of the rapid vegetative propagation methods of many plants and agricultural crops (Zimmerman, 1991; Janick et al., 1996; Gazal, 1997; AL-Hussaini, 2001) in which large numbers of plants are produced in a short period of time as well as production throughout the year in small areas compared to traditional methods, also considered as a way to solve some of the problems facing agriculture. PTCT with irradiation have been employed to improve plant traits by introducing mutations, as an effective and complementary method of traditional breeding, but is not a substitute, as well as an easy way to increase the variations and the process of screening and selection for many generations to isolate plants and cultivate, evaluate them in a field. Different materials were used (stem cuttings, leaves, seeds, embryos and callus) to study the effects of radiation on vegetative traits (AL-Tikriti, 2001; AL-Salihi, 2002; Abdul-Hussain, 2004; AL-Hussaini et al, 2010; AL-Hussaini, 2016). Gamma rays are one of the most common mutants to obtain mutations (Van Harten, 1998), as Co^{60} and Cs^{137} are the essential sources of gamma rays

(Abdul-Hussain, 2004; AL-Hussaini, 2016; AL-Tikriti, 2001).

Royal Gala is one of the most important commercial varieties, a hybrid of the Kidds orange red and Golden delicious, whose origin related to New Zealand and has spread to the United States of America, which has expanded its production since 1970 until now. Its fruits are rich in vitamins, especially vitamins A and B. Most fruits are golden yellow, interspersed with red ribbons, which are consumed in eating or exporting (Produce oasis, @1996-2011). Previous studies did not mention of the effect of re-irradiation on the behavior and response of plants to this effect. Therefore, this research was carried out to study the behavior and response of apple explants under Re-irradiation as well as the study of vegetative characterize (number of shoots and plant height), Which are researchers in plant tissue culture laboratories works to improve them and find variations that are made for getting the desired traits.

Materials and methods

Apple variety (Royal Gala) was micropropagated on MS (Murashige and Skoog, 1962) nutrient medium; pH was adjusted to 5.7 prior to autoclaving at 121ºC for 20 minutes. Apple plantlets of the Royal Gala were exposed to different doses (0, 10 ,15, 20, 25, 30 and 40 Gy) of Gamma rays with Re-irradiation twice (one week between them) (Fig. 1a). Cuttings (with 2 nodes) were excised and placed in test tubes (25x150 mm) containing MS basal medium with 3% sucrose, 7% agar and 0.1, 100, 0.5, 0.5, 0.5, 1, 0.1 mg L^{-1} of Thiamine –HCl, Inositol, Glycin, Nicotinic Acid, Pyridoxine-HCl, Benzyl adenine, Indole butyric acid, respectively. Leaf segments were excised from twice Re-irradiated plantlets and irradiate directly for one time with the same doses (Fig. 1b) placed in Petri dishes (9 cm) containing MS basal medium with 3% sucrose, 2.5% phytogel and 0.1, 100, 0.5, 0.5, 0.5, 1, 0.1, 0.2, 3, 400 mg L^{-1} of Thiamine –HCl, Inositol, Glycin, Nicotinic Acid, Pyridoxine-HCl, Naphthalene acetic acid (NAA), Thidiazuron (TDZ), Cefotoxime, respectively. Thidiazuron and Cefotoxime cold sterilized and added to the medium. The cultures were incubated in the growth room chamber at 25°C±2 under 16 h light and 8 h dark. Data of % Survival, number of shoots/ explant, plant height were taken.

The percentage of reduction in the number of shoots and plant height was calculated as follows:

$$\% \text{ reduction} = [(T1-T2) / T1]*100$$

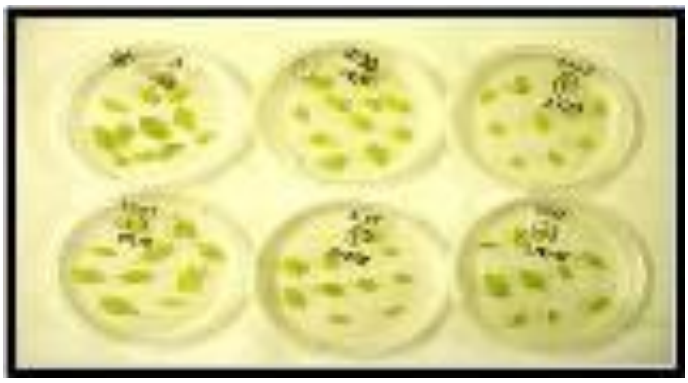
Where T1 refer to value of Re-Irradiated one time treated and T2 refer to value of Re-Irradiated twice treated. While the percentage of reduction in the number of shoots of the leaf segments which irradiate directly for one time was calculated as follows:

$$\% \text{ reduction} = [(T1-T2) / T1]*100$$

Where T1 refer to value of non-irradiated (control treatment) and T2 refer to value of irradiated treated.

[(The results were statistically analyzed using GenStat program and means were separated using L.S.D test at a probability level of 5%.

(a)



(b)

Fig. 1 (a) Re-irradiation of the plantlets of Royal Galla apple with a different dose of gamma rays. **(b)** Direct irradiation of the leaf segments of the Royal Galla Apples.

Results and discussion

Effect of Re- and direct irradiation on % survivals

Results in the table 1 showed that There was a significant decrease in the survival percentage of stem cuttings by increasing irradiation doses at re-irradiated twice to 10% at 25 Gy, whereas doses 30 and 40 Gy caused completely failed to grow. On the contrary, survival percentage was not affected by the increase of radiation doses during irradiation for 0ne time reached 100% at all doses.

Table1. Effects of Re- irradiation on % survival of stem cuttings and leaf segments of apple after 30 days.

Gamma dose Gy	% Survival			
	Stem cuttings		Leaf segments	
	Re- irradiation		Re- irradiation	
	1	2	1	2
0.0	100	100	98.0	98.0
10	100	100	94.0	88.0
20	100	100	90.00	42.0
25	100	10	88.0	18.0
30	100	0	48.0	2.0
40	100	0	24.0	0.0
	L.S.D at 0.05 = 81.01		L.S.D at 0.05 = 35.03	

The result for re- irradiation of leaf segment Table 1. Showed a gradual and sharp decline in the survival percentage by increasing radiation doses when re-irradiation twice compared with once, specifically 25 and 30 Gy reached 18% and 2%, respectively. While 40 Gy Caused a total failure in the growth of leaf segments when compared with irradiated for one time.

While, the direct irradiated of the leaf segments (Table 2) showed clear effects due to irradiation, which negatively affected their survival percentage. The lowest survival percentage was 14 and 6% at 30 and 40 Gy, respectively, compared with control (non-irradiated) and 10 Gy Which had a survival percentage 100 and 98%, respectively.

Table 2. Effect of direct irradiation for one time on the % survival leaf segments after 30 days.

Gamma dose Gy	% Survival
0.0	98.0
10	100
20	88.0
25	70.0
30	14.0
40	6.0
L.S.D at 0.05 = 43.71	

Several previous studies have addressed the effects of the use of radiation on various variations, pointing to the direct and indirect biological effects, Direct once causing a change in the genetic code or cell death. While the second, caused decomposition of water and be free radicals (Kovacs and Keresztes, 2002). The damage caused by repeated irradiation of plant for twice was significantly higher than irradiation for one time, it may be explained based on the cumulative effect of radiation dose in causing that damage. As well as the obvious impact of leaf segments, whether to excise from re-irradiated plants or directly irradiated, which unable to repair damage to cells under the influence of radiation. Or may be explained in the internal content of their growth hormones (Carpato et al., 1995) through the effect of radiation on the reduction of the amount of internal growth regulators, especially cytokines, as a result of their destruction or lack of manufacturing with the presence of irradiated (Omar et al., 1993).

Effect of Re- and direct irradiation on vegetative characteristics

The results in Table 3 appeared In general, the gradual decrease in the number of shoots and plant height of the stem cuttings by increasing radiation doses for both re-irradiation twice and once. With a note that twice Stimulate an increase in the number of shoots at doses 10 and 20 Gy, which reached 4.20 and 4.3 shoot/ stem cutting, respectively, compared to irradiation once (3.90 and 3.30 shoot/ stem cutting). The plant height of the shoots has been negatively affected by re-irradiation

twice, especially in the 25 and 30 Gy (1.41 and 0.14 cm, respectively) while the 40 Gy caused total death of the stem cuttings.

In the study of the percentage of the reduction in these characterizes, it appears from the Table 3 that it was highest at 30 Gy (86.36 and 93.12% in the number and plant height, respectively) and lowest at 20 Gy (2.61 , -30.30 % for both the number and plant height, respectively), While at 40 Gy appeared the negative effect of re-irradiation Through a sharp decline in this percentage reached 100% for both characterizes.

Table 3. Effects of Re- irradiation on number and plant height of stem cuttings after 30 days.

Gamma doses Gy	Number of shoots		% Reduction	Plant height (cm)		% Reduction
	Re- irradiation			Re- irradiation		
	1	2		1	2	
0.0	5.00	5.00	0.00	2.74	2.74	0.00
10	3.90	4.20	-7.69	3.90	2.22	43.07
20	3.30	4.30	-30.30	1.91	1.86	2.61
25	2.80	2.40	14.28	1.47	1.41	4.08
30	2.20	0.30	86.36	1.31	0.09	93.12
40	1.20	0.00	100	1.17	0.00	100
	L.S.D at 0.05 = 1.26			L.S.D at 0.05 = 0.49		

The results in Table 4 showed that the re- irradiation twice and once caused a gradual decrease in the number of shoots with the apparent negative impact of re-irradiation twice compared to once, especially, 25 and 30 Gy reached 0.55 and 0.02 shoot/leaf segment, respectively. While, 40 Gy failed to form shoots from the leaf segments. The dose 10 Gy given highest number of shoots (27.07 shoot/leaf segments) compared with other doses (Fig. 2). The percentage of reduction of shoot formation decreased by increasing the doses of radiation reached to 100% at 40 Gy.

Table 4. Effects of Re- irradiation on number of shoots of leaf segments after 30 days.

Gamma doses Gy	Number of shoots		% Reduction
	Re-irradiation		
	1	2	
0.0	32.32	32.32	0.00
10	27.07	12.29	54.59
20	16.86	1.05	93.77
25	14.68	0.55	96.25
30	3.17	0.02	99.36
40	0.60	0.00	100
L.S.D at 0.05 = ?????			

Fig. 2: Shoots formation from leaf segments of Royal Galla Apple.

The irradiated dose had a clear effect on the number of shoots of leaf segments, which irradiated directly for one time (Table 5), that caused a gradual decrease by increasing radiation doses comparable with control treatment (non-irradiated), specifically at 30 and 40 Gy (0.50 and 0.07 shoot /leaf segment, respectively) While, 10 Gy given the highest number of shoots (21.99 shoot / leaf segment) compared to other doses. As for the reduction in shoots formation, it was increased by increasing the doses compared with control treatment.

Table 5. Effect of direct irradiation for one time on the number of shoots of leaf segments after 30 days.

Gamma doses Gy	Number of shoots	% Reduction
0.0	32.32	0.00
10	21.99	31.96
20	11.56	64.23
25	7.89	75.58
30	0.50	98.45
40	0.07	99.78
L.S.D at 0.05 = 4.23		

It is clear from the results that the effect of irradiation and frequency on vegetative characteristics were different in their effect depending on the type of explants. It was observed that the stem cuttings were less affected by radiation compared with leaf segment, whether re-irradiated or directly, it has been explained previously based on the leaf content of growth hormones. The decrease in the number of shoots and plant height by radiation is due to several explanations, including the decrease in cell growth and division due to the harmful effect of radiation on the efficiency and activity of the cells as well as the effect of protein synthesis (Grosch, 1968; Gunckle and Sparrow, 1961) or the effect of radiation on the chromosomes inside the nucleus of the cell, which produces nucleic acids and formation of new enzymes does not perform the function of the original enzymes in controlling the biological processes within the cell (van Harten, 1998; Maliga, 1980). It was also observed that Low doses, specifically 5 and 10 Gy have no effect on the vegetative growth compared to other doses, may be explained by the breakdown of enzyme inhibitors in the cell. This causes an increase in cellular activity (FAO/IAEA, 1977).

Conflict of interest statement

Authors declare that they have no conflict of interest.

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