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INFLUENCE OF GAMMA RADIATION ON *IN VITRO* GROWTH MICROTUBERSATION AND HORMONAL CONTENT OF SOME POTATO (*SOLANUM TUBEROSUM* L.) CULTIVARS

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Abstract

Gamma irradiation, which is a mutagen used in vegetable and crop plant breeding is expected to induce novel mutations and alteration agents in developing a genetic variety. The objective of this study was to assess the vegetative growth, plant hormonal parameters and yield of three cultivars of potato (*Solanum tuberosum* L. cv Arnova, Lizeta and Safari) which were irradiated with different doses of gamma rays (0, 2.5, 5, 10, 15 and 20 Gy) respectively, using *in vitro* techniques. The results of the experiment showed that Arnova was significantly superior in plant height, number of leaves, microtubers fresh weight, IAA and GA hormones content. Furthermore, the microtubers reached (7.25 cm, 10.5 leaf, 0.4 g, 0.673 $\mu g.g^{-1}$ and 1.3 $\mu g.g^{-1}$ in microtubers) respectively per plant. The total yield per plant and the percentage representing the number of tubers with the size 1.5-2 cm, reached (9.3, 26.56, 6.34 % and 47 %) respectively. However, Lizeta significantly differed from Arnova and Safari in the total yield which reached (0.72, 0.55, and 0.62 g) respectively. The gamma radiation (2.5 Gy) increased the average plant height significantly, and the number of shoots and tubers were determined to have a significant increment ratio (1.86 %, 19.04 %) which was the average compared to the control treatment. The interaction of the Arnova and 2.5 Gy was significantly superior in the total yield reaching (0.9 g per explant), while Lizeta at 2.5 Gy significantly superior in a number of tubers reaching 2.8 tubers per explant. From the previous results, the best growth was observed in the total yield of microtubers at the dose 2.5 Gy in Arnova or Lizeta cultivar.

Key words : Irradiation, microtubers, cultivars, Lizeta, Arnova, Safari,plant tissue culture,biotechnology.

Abbreviations: ABA - abscisic acid; BA -6-Benzylaminopurine ;GA3- Gibberellin;IAA- Indole-3-acetic acid; MS - Murashige and Skoog

Introduction

Many people globally rely on potato *Solanum tuberosum* L. as a major food source which is rich in carbohydrates and starch. As a food source, potato provides about 76 calories and contains many vitamins, including vitamins B, C and salts (Zamotaeva, 1997). However, despite the increase in the production of potatoes in Iraq from 204,597 to 580,000 tons during the period 2010 to 2013, the World Food Organization (FAO), indicated that this level of productivity would not sufficiently meet the needs and demands of the local market and its people. Moreover, this has necessitated the need to import produce from Arab and global markets to compensate for the large deficit and shortage. Indeed, there is an urgent need to promote agricultural policies towards the optimal utilization of agricultural resources and to encourage the adoption of modern agricultural technologies. Accordingly, this would enable the cultivation of high-quality plant varieties to be further expanded as well as adopting better breeding methods to improve the overall quality through the development of new genetic structures and selection of desirable structures with high productivity and multiplication. Mutation is among the most significant nuclear agriculture techniques used extensively for yield enhancement to achieve high sustainability. The technologies to improve radiation provide the most efficient methods to induce plant mutation and enhance plant character. This is because novel varieties of food crops subjected to induced mutations have helped to significantly increase crop

production in areas that are accessible to most people (Suprasanna *et al.*, 2014). Also, these modifications occur randomly, and the stability relies on cell damage following irradiation at molecular levels. Mutation breeding using nuclear technology acts as a complementary method to cross breeding and hybridization in enhancing variability for crop improvement, thus generating genetic variations. Indeed, this variation could provide better crop characteristics that could benefit farmers and improve their standard of living. Previous research activities in mutation have reported the survival rate, tiller production and the number of filled grains as important traits affected by mutagens treatment (Abdul Haris *et al*., 2015). Nonetheless, by exposing crops to mutagen with optimal radiation, this could help to improve crop varieties and assist breeders in the longer term to improve the crops characteristics. *In vitro* techniques are recognized as a valuable, if not significant source of supply for modern plant enhancements and in development programs to present new traits in plants, to increase elite collections and to advance suitable cultivars in the least time (Kadhimi *et al*., 2014). Plant tissue culture is best described as a process that comprises the growth and multiplication of plant cells, where the technology is established on micropropagation, and through which rapid growth and reproduction is accomplished from small stem cuttings, axillary buds, and to some extent from cell clumps in suspension cultures, bioreactors and somatic embryos (Al-Safadi *et al*., 2000). Furthermore, using *in vitro* methods enables the mechanism for generating sizeable plant populations to induce mutation, selection, and quick multiplication of selected mutants. Notably, well-established clones can be upgraded by modifying certain properties via mutation induction. Irradiation of micro-propagated plants and somatic embryos produce a small scaled-down representation of trees and seeds in a petri dish rather than in the actual field. Therefore, a coordinated research project was initiated regarding *in vitro* techniques to select radiation-induced mutations that have managed to adapt to complex and challenging environments. This research study aims to use the radiation of gamma-rays by CO60 for three cultivars of potatoes to study their growth and the formation of microtubers *in vitro*.

Materials and Methods

The present study was carried out at the Tissue Culture Laboratories of Genetic Engineering Department and the Biological Control Department of the Agricultural Research Directorate, Ministry of Science and Technology, Iraq, between December 2017 and April 2018.

The experimental materials consisted of potato *Solanum tuberosum* L.cv Arnova, Lizeta and Safari class Super Elit. The materials were irradiated with different doses of gamma rays (0, 2.5, 5, 10, 15, and 20 Gy) respectively, using *in vitro* technique.

Next, meristems were obtained from uniform growing sprouts (~ 2 cm long) established from three potato cultivars (tubers) Arnova, Lizeta and Safari class Super Elit. Sterilisation is an important aspect of tissue culture because tissue culture targets *in vitro* propagation of selected plant material that is free from of contamination of any other microorganism. Therefore, if appropriate measures are not adopted, the cultures may become contaminated by microorganisms. Also, using sterilised instruments is an important aspect of maintaining an aseptic environment for tissue culture. The sprouts were first cut from sterilised sprouting potato tubers, which were initially washed using distilled water before further washing with Clorox (Sodium hypochlorite 10%) three times for 15 min for internal surface sterilisation and then washed five times in distilled water in a laminar air flow cabinet. The sterilised sprouts were then cut to isolate the apical meristems which were then cultured on shoot induction full-strength MS medium (Murashige and Skoog, 1962) (table 1). Different combinations of plant growth regulator medium were used for tuber sprouting (table 2), Upon obtaining the full number of plantlets, these were then transferred into microtuber information media (table 3) and the pH adjusted to 5.7 using 1N NaOH or 1N HCl. Lastly, the final volume was brought up to 1000 ml with distilled water and $7g.l^{-1}$ agar type (Agar-Agar) was added. The media was next sterilised via autoclaving at 121°C and 15 lbs inch-2 for 25 min. The sterilised medium was then allowed to cool at ambient room temperature.

This was followed by quantitative analysis of zeatin activity using the method of Unyayar *et al*. (1996). The IAA and GA_3 concentrations were determined based on the methods of Nuray *et al.* (2002) and the quantitative analysis of the ABA content was performed using the method of Hein *et al.* (1984).

Statistical analysis

The factorial-type experiment was based on C.R.D, and the factors included gamma irradiation and cultivars. Also, this study involved three replications. A normality test was carried out on the data before performing the analysis of variance using SPSS software (SPSS Version 19). Significant differences were determined from among the mean values of treatments by employing the least significant differences (LSD) test, at 0.05 level.

Table 1 : MS medium components used as stock solutions for plant tissue culture experiments.

Macronutrients		
Components	**Chemical formula**	**Weight ($mg.l^{-1}$)**
Ammonium Nitrate	NH_4NO_3	1650
Potassium Nitrate	KNO_3	1900
Calcium Chloride Anhydrate	$CaCl_2.2H_2O$	440
Magnesium Sulphate Anhydrate	$MgSO_4.7H_2O$	370
Potassium Phosphate Monobasic	$KH_2.PO_4$	170
Micronutrients		
Boric Acid	H_3BO_3	6.20
Potassium Iodide	KI	0.83
Manganese Sulphate. $4H_2O$	$MnSO_4.4H_2O$	22.30
Zinc Sulphate. $7H_2O$	$ZnSO_4.7H_2O$	8.60
Molybdic Acid (Sodiumsalt).$2H_2O$	$Na_2MoO_4.2H_2O$	0.25
Cupric Sulphate. $5H_2O$	$CuSO_4.5H_2O$	0.025
Cobalt Chloride. $6H_2O$	$CoCl_2.6H_2O$	0.025
Chelated Iron		
Sodium Ethylene Diamine Tetraacetate	Na_2-EDTA	33.6
Ferrous Sulfate. $7H_2O$	$FeSO_4.7H_2O$	27.8
Vitamins		
Thiamine. HCL	$C_{12}H_{17}CIN_4OS.HCL$	0.1
Nicotinic Acid (Free Acid)	$C_8H_{11}NO_3.HCL$	0.5
Pyrodoxine. HCl	$C_6H_5NO_2$	0.5

Results and Discussion

Plant height

The results showed that all the irradiation doses with gamma-rays were effective in controlling plant height, and the treatment of 20 Gy, produced the lowest plant height of 4.7 cm. However, there were noticeable effects for the cultivars regarding plant height; Arnova cultivars provided the highest average for plant height reaching 7.25cm (table 4). The interaction analysis between the percentage of cultivars and irradiation dose determined that the lowest plant height was 3.16 cm in the Lizeta cultivar, with 20 Gy, whereas the highest plant height was 7.78 cm in the Arnova cultivar with 2.5 Gy.

Number of shoots

The cultivars all responded differently regarding the number of shoots (table 5). The Safari cultivar was shown to be significantly higher than the other cultivars, which gave 2.15 shoots per plant. The results with 2.5 Gy displayed significantly higher shoots per explant, which gave 2.75 shoots per explant. Furthermore, the interaction analysis between the cultivars and gamma-rays observed that Lizeta in 2.5Gy gave the highest number of shoots and the lowest was number was for Lizeta in 20Gy reaching (3.34 and 1.09)shoots per plant, respectively (table 5).

The gamma-ray treatments further showed the lowest number of leaves, whereas the highest number was 9.92 leaves per explant under control treatment (table 6). The results of the Arnova showed the significantly higher number of shoots than the Lizeta and Safari cultivars which gave the values 10.50, 7.88 and 5.75 leaves per explant, respectively. The interaction analysis between the cultivars and gamma rays also showed that Lizeta explants, which were grown free from gamma rays produced the highest number of leaves, with the lowest number observed for the Safari explants with 20 Gy reaching (13.7, 3.2) leaves per explant, respectively (table 6).

Diameter of the microtuber

The results in table 7 for Lizeta cv. showed the largest diameter of the microtuber was 0.70 cm, but for Arnova cv, it showed that the lowest diameterwas only 0.47cm. Moreover, the results showed that there were no significant differences between the dose of gamma rays in the diameter of the microtuber. The interaction analysis between the cultivars and gamma-rays showed that the

Table 2 : Medium components used as stock solutions for sub culture of plant tissue culture experiments.

Components	Weight ($mg.l^{-1}$)
Murashige and Skoog Salt	Full strength
Sucrose	30000.00
Inositol	100.00
Thiamine - HCl	0.40
Glycine	2.00
Nicotinic acid	2.00
Indole acetic acid (IAA)	1.00
Agar	6000.00

Table 4 : The effect of gamma radiation on the plant height (cm).

Mean	Cultivars			Doses Gy
	Safari	Lizeta	Arnova	
6.55	5.13	7.28	7.25	0
6.43	6.11	5.42	7.78	2.5
5.85	5.9	4.97	6.68	5
5.55	6.77	4.53	5.35	10
5.59	6.2	4.66	5.93	15
4.7	5.23	3.16	5.71	20
	5.89	5.00	7.25	Mean

L.S.D. 0.05, Cultivars = 0.21, interaction = 2.15, Doses = 1.04

Table 5 : The effect of gamma radiation on the number of shoots per explant.

Mean	Cultivars			Doses Gy
	Safari	Lizeta	Arnova	
2.31	2.30	2.40	2.23	0
2.75	2.78	3.34	2.12	2.5
1.81	1.53	2.12	1.77	5
1.85	2.11	1.65	1.78	10
1.67	2.42	1.20	1.40	15
1.42	1.80	1.09	1.38	20
	2.15	1.96	1.78	Mean

L.S.D 0.05, Cultivars = 0.21, interaction = 0.51, Doses=0.29

lowest diameter was 0.30cm in Arnova with 20Gy, whereas the largest diameter was 0.88 cm in Lizeta with 10Gy.

Microtuber numbers

The results presented in table 8 show that Lizeta had the highest number of microtubers, 1.94 tubers per explant, which was significantly higher than for the other cultivars. The treatment with 2.5Gy provided the maximum number of microtubers with an average of 2.47 tubers per explant, which was significantly different from the other doses.

Table 3 : Medium components used as stock solutions for microtubers information.

Components	Weight ($mg.l^{-1}$)
Murashige and Skoog Salt	Full strength
Sucrose	60000.0
Inositol	100.0
Thiamine - HCl	0.4
Glycine	2.0
Nicotinic acid	2.0
Indole acetic acid (IAA)	1.0
Benzyl adinine (BA)	1.5
Agar	7000.0

The interaction analysis between the cultivars and gamma-raysalso showed that Lizeta with 2.5Gy produced the highest microtuber number and the lowest number was for Safari with 20Gy reaching (2.80, 1.12) tubers per explant, respectively.

Fresh weight of microtuber and total yield

Accordingly, the results displayed in table 9 shows that the Gamma-ray at 10 Gy exhibited the maximum value 0.4 g of microtuber fresh weight. However, there were no significant differences observed between the control, 2.5 with 5Gy, which produced values of 0.34, 0.35 and 0.37 g, respectively.

The results also indicated that the cultivar of Lizeta was highly significant compared to the other cultivars, which only produced the value of 0.40 g (table 9). Furthermore, the interaction analysis performed between Safari and the dose of 10 Gy was highly significant in the fresh weight of the microtuber compared with other treatments, which only provided a value 0.47 g, while the Arnova treated with 15 Gy gave the lowest value of 0.18 g.On the other hand, the results showed (table 10) that the cultivar of Lizeta was highly significant compared to the other cultivars in total yield per explant, giving the value of 0.72 g.

The results indicated that with 2.5 or 5Gy, this was found to be highly significant as compared to the other investigated precursors in a total yield of microtuber per explant, which gave a value of 0.85 mg for each, while for 20 Gy this produced the lowest value of 0.36g of the microtubers dry weight. The interaction analysis of the Arnova or Lizeta cultivars treated with 205Gy showed highly significant differences to the other treatments in total yield characteristics, which provided the value of 0.98 and 0.94 g, respectively. Whereas, the Arnova with 15Gy gave the lowest value of microtuber dry weight 0.23 g (table 10).

Table 6 : The effect of gamma radiation on the number of leaves per explant.

Mean	Cultivars			Doses Gy
	Safari	Lizeta	Arnova	
9.92	8.85	13.7	7.2	0
8.58	9.88	9.3	6.55	2.5
8	11.29	6.37	6.34	5
8.15	11.25	7.15	6.04	10
7.9	12.45	6.11	5.14	15
5.72	9.3	4.65	3.2	20
	10.50	7.88	5.75	Mean

$L.S.D_{0.05}$, Cultivars 0.36, Interaction = 0.89, Doses = 0.51

Table 7 : The effect of gamma radiation on the diameter of microtuber (cm).

Mean	Cultivars			Doses Gy
	Safari	Lizeta	Arnova	
0.49	0.42	0.55	0.51	0
0.54	0.42	0.67	0.52	2.5
0.59	0.51	0.69	0.58	5
0.67	0.63	0.88	0.49	10
0.64	0.71	0.80	0.41	15
0.50	0.62	0.59	0.30	20
	0.55	0.70	0.47	Mean

$L.S.D_{0.05}$, Cultivars = 0.23, Interaction = 0.33, Doses = N.S

Table 8 : The effect of gamma radiation on the number of microtubers per explant.

Mean	Cultivars			Doses Gy
	Safari	Lizeta	Arnova	
2.20	1.92	2.40	2.20	0
2.47	1.85	2.80	2.76	2.5
2.30	2.50	2.50	2.01	5
1.48	1.49	1.45	1.50	10
1.32	1.32	1.30	1.33	15
1.22	1.12	1.38	1.16	20
	1.70	1.94	1.86	Mean

L.S.D 0.05, Cultivars = 0.05, Interaction = 0.19, Doses = 0.06

Therefore, this may increase the biosynthesis of chemical compounds in the photosynthesis process and increase vegetative growth (tables 4 and 5). Indeed, this leads towards increasing the number and yield (tables 8 and 9) of the tubers and increasing the tissue content of protein or growth hormones and increasingthe tubers' weight and total yields (Issa *et al.,* 2008).

Plant hormonal parameters

The results of the experiment indicated that both the growth and yield of potato are affected by the combination of growth regulators and plants, as with another cultivar (Sheeba *et al*., 2013). The cultivar of Arnova provided the highest IAA content (fig. 1) and GA content (fig. 1), which reached 0.673 and 1.3 $\mu g.g^{-1}$, respectively, but the cultivar of Safari produced the highest Zeatin (fig. 3) reaching 0.698, 3.354 $\mu g.g^{-1}$, respectively, while the cultivar of Lizeta produced the highest ABA (fig. 4). Significant differences were also recorded among the dose of gamma-ray treatments in their effect on hormone concentrations; 2.5 Gy produced the highest impact on the rate of GA (fig. 2), Zeatin (fig. 3) and ABA (fig. 4) reaching 1.444, 3.796 and 0.814 $\mu g.g^{-1}$, respectively, as compared with the other doses. The results also indicated that the control showed significantly higher IAA content giving 0.570 $\mu g.g^{-1}$ (fig. 1).

Table 9 : The effect of gamma radiation on fresh weight of microtuber (g).

Mean	Cultivars			Doses Gy
	Safari	Lizeta	Arnova	
0.34	0.34	0.34	0.35	0
0.35	0.35	0.38	0.31	2.5
0.37	0.42	0.41	0.27	5
0.40	0.47	0.45	0.29	10
0.30	0.31	0.42	0.18	15
0.30	0.26	0.39	0.25	20
	0.36	0.40	0.28	Mean

L.S.D 0.05, Cultivars = 0.05, Interaction = 0.18, Doses = 0.09

Table 10 : The effect of gamma radiation on total yield of microtubers (g) per explant.

Mean	Cultivars			Doses Gy
	Safari	Lizeta	Arnova	
0.75	0.67	0.91	0.68	0
0.85	0.63	0.94	0.98	2.5
0.85	1.05	0.82	0.67	5
0.60	0.70	0.67	0.42	10
0.40	0.41	0.56	0.23	15
0.36	0.29	0.45	0.34	20
	0.62	0.72	0.55	Mean

L.S.D 0.05, Cultivars = 0.13, Interaction = 0.78, Doses = 0.15

The interaction analysis between the cultivar and gamma-rays showed that the Lizeta cultivar with 2.5Gy produced the highest content of ABA and Zeatin reaching (0.931, 4.518 $\mu g.g^{-1}$), respectively.

Notably, low-dose irradiation will induce growth stimulation by changing the hormonal signalling system in the plant cells or by increasing the antioxidative capacity

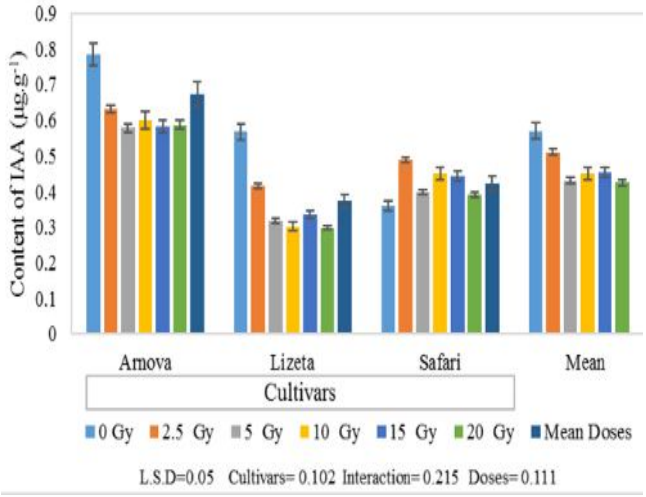

Fig. 1 : The effect of gamma radiation on IAA content in microtuber.

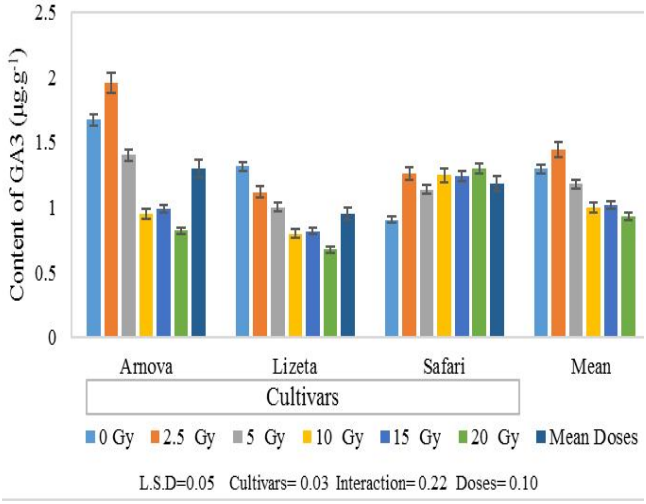

Fig. 2 : The effect of gamma radiation on GA_3 content in microtuber.

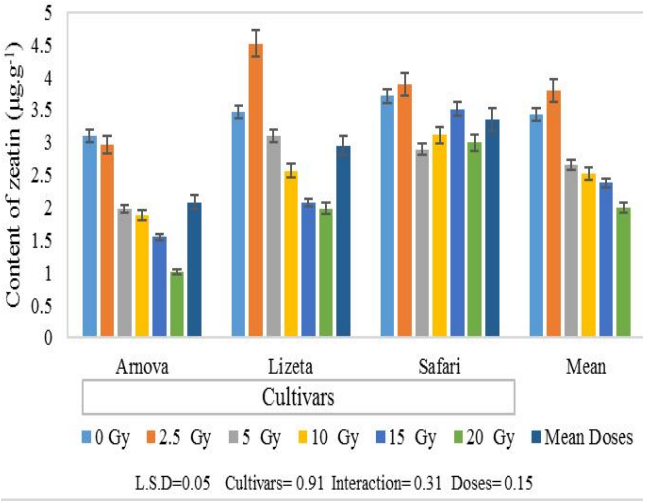

Fig. 3 : The effect of gamma radiation on zeatin content in microtuber.

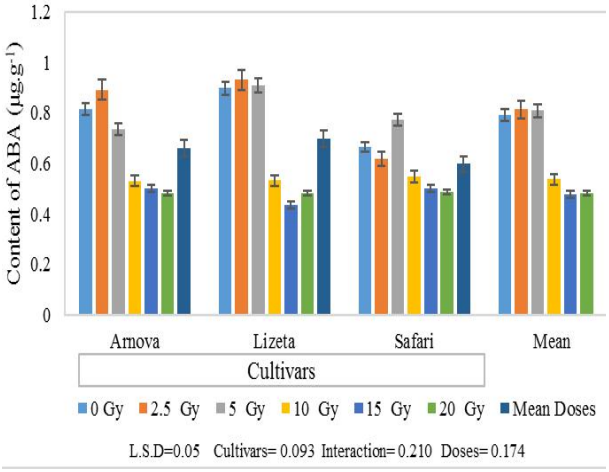

Fig. 4 : The effect of gamma radiation on zeatin content in microtuber.

of the cellseasily to, therefore, overcome daily stress factors such as the fluctuations of light intensity and temperature in the growth condition (Wi *et al*., 2007). Indeed, this could occur because the hormetic effects of low-dose ionising radiation on plants include quicker cell proliferation and motivated germination.

The results of this study have demonstrated that the reduced development in height was observed in the plantlets, due to enhancing the ion doses applied to the plants. Ling *et al*. (2013) indicated that irradiation caused a decrease in plantlet elongation through carbanions. Masuda *et al*. (2002) in a separate study also reported areduction in radical elongation caused by the treatment of tomato seeds with carbon and helium ions.

Furthermore, Preuss and Britt (2003) reported that irradiation could seriously affect the cell cycle captured at the G2/M stage through the effect of genome or somatic cell division, therefore inhibiting development. Jones *et al*. (2004) further reported that the adverseeffect of radiation on plants could be indirectly affected by metabolic variations over free fundamental development as well as by DNA injury of the isolated cells.

Abdul Haris *et al*. (2015), established that the morphological characters with a gamma radiation dose were affected in relation to the average plant height. Therefore, it is described that mutation is most easily seen in the case of changes in colour, size, or shape between non-irradiated and irradiated plants.

Therefore, by introducing ionising radiation to a cell culture, this can induce a wide range of molecular injuries, including DNA damage, mutation, chromosomal rearrangement, and cell inactivation (Nishiguchi *et al*.,

2012). The gamma rays used in this process produced free radicals when interacting with molecules in the cell. Indeed, these free radicals damaged the components of the plant cells and influenced the biochemical, physiology and anatomy of the plant (Zamotaeva, 1997). Notably, low mutagen doses increased the fresh weight of the plant.

All researchers indicated that, cultivars have different potential in vegetative growth and the production of microtubers (Al-Safadi *et at*., 2000; Al-Salihi, 2002 and Issa, 2006) and possibly, the genotype in cultivars, resulting in different yields due to the capacity of the genotype in the production of the endogenous levels of growth, which regulatesas well. This is also due to the increase in the cytokinin rate (fig. 3), which is the primary factor of microtuber initiation (table 10). Indeed, the effect of zeatin on micro-tuberisation may also result from the relationship with ethylene hormone biosynthesis which influenced microtuber initiation (Romanov *et al*., 2000). The obtained results, therefore, recommend the possibility of controlling the best growth vegetative, number and total yield of microtubers using 2.5 Gy in Arnova or Lizeta cultivars.

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