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Research Article

ACCELERATING MICROTUBERIZATION IN POTATO BY IRRADIATION

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ABSTRACT

This study was carried out to investigate the stimulating effects of low doses of gamma irradiation on microtuber production. The etiolated shoots of potato were cultured on simple MS medium. The nodal segments from these seedlings were sub-cultured on MS basic medium that was supplemented with 0.8% agar, 6 mgL^{-1} BAP, 4 mgL^{-1} kinetin and 60 gL^{-1} sucrose and then were irradiated with 4 doses of gamma radiation (1, 3, 5, 7 Gray). The acceleration of plantlets growth and microtuber production were examined. The experiment was laid out in Completely Randomized Design (CRD). The data collected were analyzed using appropriate statistical technique which showed that irradiation doses had significant effect on microtuber formation and 3-Gray gamma rays produced best results.

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Basic problem in the production of potato is the unavailability seed with good quality. Imported potato seed is used to minimize the threat of potato seed born and viral diseases like potato leaf roll virus (PLRV), potato virus Y (PVY) and potato virus X (PVX). The viruses are the major cause of degeneration of seed and results in severe yield losses (Hooker, 1986; Lazar and Georgescu, 1987). For a virus free seed supply system, one needs to produce pre-basic seed as microtubers in test tubes and multiply them in green house for production of minitubers. The microtubers can be planted in screen house to produce pre-basic seed (Abbott and Belcher, 1986).

Production of virus free pre-basic seed by tissue culture techniques are used worldwide to produce pre-basic, virus-free seed potatoes known as microtubers. Plant tissue culture is the only technique that can eliminate approximately 100% viruses in seed production programs and microtuber is one of the strategies in this perspective. Because of their small size and weight, microtubers have tremendous advantages in terms of storage, transportation and mechanization.

They can be directly sown into the soil and can be produced in bulk in any season. They have the similar morphological and biochemical characteristics to field produced tubers. Therefore, mass production of potato microtuber is likely to revolutionize the world potato production. A number of research groups all over the world are trying to bring about this revolution (Sakha *et al.*, 2004; Gopal *et al.*, 2004; Zhijun *et al.*, 2005).

The recent advancement in tissue culture and the flexibility of organ development in potato allows for alternative methods of propagation through *in vitro* techniques. In many countries these techniques have boosted first multiplication steps in seed production programs by using *in vitro* plantlets, microtubers (Bizarri and Ranalli, 1995).

In the rapid multiplication of clean material *in vitro*, the use of single nodal cutting is the most preferred method of propagation since it ensures higher propagation rates with maximum genetic uniformity in potato (Chandra and Naik, 1993). Tissue culture techniques has its proven ability to successfully induce invitro growth and development (callogenesis and organogenesis) on a nutrient supplemented medium (Rafique, 2012; Rafique, 2012;Rasheed, 2013). So, evaluating radiatios effect on plant growth and development was much needed.

Low doses of γ-radiation have been reported to slightly stimulate plant growth and development, and improve the yields and qualities of plants *in vivo* (Wiendl *et al.*, 1995) and *in vitro* (Al-Safadi and Simon, 1990), apart from its induction of mutants in flower colour and shape (Misra *et al.*, 2003). To date, most of the reports about gamma irradiated potato were focused on the characteristics of the process and storage period (Ciesla and Eliasson, 2002; Wang and Chao, 2003). Limited information has been reported on the influence of γ-radiation on the potato production and quality development, especially on potato micropropagation material (microtubers).

The purpose of this work was to investigate the stimulating effects of low doses of gamma radiation on the development, yield and quality of microtubers and to determine the effect of different doses of raditions on invitro growth and development in potatoes.

MATERIALS AND METHODS

The present research was carried out in Plant Tissue Culture Cell, Institute of Horticultural Sciences, University of Agriculture, Faisalabad during the years 2008-2010. Potato varieties Cardinal, Diamant and Desiree were obtained from Plant Virology Section, Ayub Agricultural Research Institute, Faisalabad. Potato tubers were surface sterilized using 5% sodium hypochlorite solution for 10 min. These tubers were washed three times with sterile distilled water and kept in craft paper bags. These bags were stored under dark conditions for two months at constant temperature of 25°C for etiolated shoot sprouts (Ahmad and Khan, 1993). The etiolated shoots were used for regeneration of *in vitro* plantlets on simple MS medium without addition of hormones. Nodal segments of *in vitro* grown plantlets were used for microtuber induction and callus formation. Etiolated shoots were removed from potato tubers and dipped in tap water for half an hour. Then the explants were surface sterilized in 70% alcohol for 2-3 minutes and for 20 minutes in 15% sodium hypochlorite with the addition of one or two drops of Tween-20. Finally explants were washed with double distilled autoclaved water. MS basic medium (Murashige and Skoog, 1962) supplemented with 0.8% agar, 6 mg/L BAP, 4 mg/L kinetin and 60 g/L sucrose. The pH was set to 5.8. This media was used for microtuber formation. After one week of culture the growing shoots were irradiated with different doses of gamma radiation (0, 1, 3, 5 and 7 Gy) to check their effect on microtuber formation and starch contents (Figure 1). The radiation facility was obtained from Nuclear Institute of Agriculture and Biology, Faisalabad. pH of medium was adjusted to 5.8. Agar was used 8 g/L of medium. In each test tube 10 mL medium was dispensed and covered with polythene covering with the help of rubber bands. The media was sterilized in an autoclave at a temperature of 121°C, under a pressure of 15 psi for 20 minutes. Hands were washed with soap and disinfected with 96% ethanol. The culture room and laminar air flow cabinet were sterilized by Ultra Violet Light for 45 minutes. All instruments were sterilized in bead sterilizer before and with 96% ethanol during cultures. The explants were cultured on media after sterilization. Cultures were incubated at 25±2 °C under 16/8 hours photoperiod (2500 lux) with white fluorescent tubes (Philips TL 40W/54). In second experiment, the cultures were kept in complete darkness at 25°C to induce callus. Data were collected regarding Shoot length (cm), Number of leaves, Number of nodes, Root number, Number of days to microtuber formation, number of microtuber per tube, weight of microtuber (mg), diameter of microtuber (mm), Vitamin C content in fresh microtubers (mg/g). Ascorbic acid was estimated by the method described by Ruck (1961). Vitamin C contents were calculated by using the following formula:

$$\text{Vitamin C (mg)} = \frac{1 \text{ x R1 x V}}{\text{R x W x V1}} \times 100$$

R_1 = mL dye used in titration

R = mL dye used in titration of 1 mL standard ascorbic acid solution (1 mL of 0.1% ascorbic acid + 1.5 mL of 0.4% oxalic acid).

V_1 = mL of juice used.

V = volume of aliquot made by 0.4% oxalic acid.

W = mL of aliquot used for titration

The experiment was laid out according to Completely Randomized Design (CRD) with five treatments and four replications (5 test tubes per replication) in first experiment and t Data was collected and analyzed by using MSTAT-C software.

Figure 1: Shoot regeneration in three varieties from etiolated sprouts on simple MS media

RESULTS AND DISCUSSION

Shoot length (cm)

The analysis of variance (Table 1) for shoot length showed highly significant results and their interactions. The means of varieties are shown in Table 2. The variety Diamant hold top position (3.91 cm) while variety cardinal (3.68 cm) is at second position and variety Desiree (3.29 cm) hold last position. First two varieties were statistically at par to each other. It is clear form Table 2 that control gave the highest (4.68 cm) shoot length which is followed by 1 Gy, 3 Gy, 5 Gy and 7 Gy doses of radiation producing 4.02 cm, 3.26 cm, 3.10 cm, 3.06 cm shoots, respectively. Last three treatments' means were similar with each other. Interaction between varieties and radiation doses were also significantly different (Table 2). The greatest shoot length 5.74 cm was produced by Diamant on control followed by Cardinal and Desiree with shoot lengths 4.74 cm (T1) and 3.57 cm (T2), respectively. All three varieties were statistically different for shoot length on control. The highest irradiation doses (5 or 7 Gy) produced the lowest shoot length in all varieties.

The results indicated that control treatment produced the highest shoot length in all three varieties. Similar results were found by Results also suggest that with the increment of radiation doses, the shoot length decreased in all three varieties, which is in accordance with the results of Al-Safadi and Simon (1990).

Number of leaves

The analysis of variance for number of leaves showed highly significant results for radiation doses and interaction between varieties and radiation doses but it showed significant for varieties (Table 1). The cultivar Desiree produced significantly higher number of leaves (4.43) which was statistically at par with Cardinal (4.33). The variety Diamant holds 3rd position in all three varieties which produced 4.06 numbers of leaves (Table 2). Table (3) shows the comparison of means for different doses of gamma radiation used. It is clearly visible that control treatment produced the highest (5.87) number of leaves than other treatments. It also shows that 3 Gy and 5 Gy treatments were statistically similar with each other. Variety Desiree (6.59) and Cardinal (6.50) produced the highest number of leaves on control and were statistically similar. The lowest number of leaves was recorded in Desiree with 7 Gy treatments and was statistically similar with other varieties. This indicates that the growth of seedlings is reduced with increase in radiation dose. These results are also confirmed by Hung and Johnson (2008), who found deformed leaves by treating with high dose of radiation.

Number of nodes

The analysis of variance for number of nodes (Table 1) showed highly significant results for radiation doses, varieties and interaction between varieties and radiation doses. The cultivar Desiree produced significantly highest number of nodes (6.86) as compared to other two varieties (Table 2). Diamant and Cardinal were statistically non significant with each other and produced 6.21 and 5.93 number of nodes, respectively. Gamma radiation doses significantly affected the number of nodes. Control treatment produced maximum number of nodes (8.75) followed by 1, 3, 5 and 7 Gy treatments which produced 6.20, 5.09, 5.60 and 5.03 number of nodes respectively (Table 3). All treatments were statistically different with each other except 1 and 3 Gy treatments which were similar with each other. Variety Diamant produced maximum (6.86) mean number of nodes on control, followed by Cardinal (6.21) and were statistically at par with each other. The minimum number of nodes in Diamant (5.15) and Desiree (6.39) and Cardinal (4.54) were produced at 7 Gy gamma radiation dose. The results of Miller *et al.* (1985) partially lines with present results.

Root number

The analysis of variance for root number showed highly significant results for radiation doses and interaction between varieties and radiation doses. The non significant results showed only by varieties as shown in Table 1. It was observed from the comparison of means of three varieties that Diamant produced maximum number of roots (1.25) followed by Cardinal (1.23) which was statistically at par with Diamant. Desiree produced the lowest number of roots (1.08) as compared to other two varieties (Table 2). Table 3 shows the comparison of means of gamma radiation doses. It was observed that only control treatment produced roots (5.97). Gamma radiations inhibited the root formation. Interaction between varieties and radiation doses showed that variety Diamant developed maximum (6.29) number of roots on control followed by Cardinal (6.17) and Desiree (5.43). The root numbers of variety Diamant and Cardinal were statistically at par at 0 Gy gamma radiations. These results showed that with the application of gamma rays, the production of roots in all three varieties inhibited. Our results are in accordance with the results of Al-Safadi and Simon (1990). They said that no roots were formed in the carrot culture with the increase in gamma radiation.

Number of days to microtuber formation

The analysis of variance (Table 1) for number of days to microtuber formation showed highly significant results for all the factors studied and their interactions. The means of varieties are shown in Table 2. The variety Desiree hold top position (22.85 days), Cardinal (21.57 days) is at second position while Diamant (18.15 days) holds last position. All varieties means are statistically different from one another. The means of varieties also shows that higher the mean values, higher are the days taken to induce first microtuber. This suggests that Diamant hold minimum mean value (18.15), which started microtuber formation earlier than other varieties. The other two varieties i.e., Cardinal and Desiree start their first microtuber after 21.58 days and 22.85 days, respectively. It is clear from mean Table 3 that 7 Gy treatment took maximum (27.67) days to microtuber induction which is followed by 0 Gy, 5 Gy, 1 Gy and 3 Gy doses of radiation taking 23.75, 21.92, 16.04 and

14.92 days, respectively. That data also shows that 3 Gy radiation dose took minimum days to microtuber formation. Table 1 also shows the interaction between varieties and radiation doses. The maximum days 31.75 was taken by Desiree at 7 Gy treatment followed by Cardinal which took 31.25 days for microtuber formation, but the variety Diamant took maximum days (22.75) on control. The variety Diamant and Cardinal were at par for days to produce microtuber on control. The data suggests that variety Cardinal took minimum days (14.75) at 3 Gy gamma radiation doses, while other two varieties i.e, Desiree and Diamant took minimum days 15.00 and 15.00 at the same treatment, respectively. Microtubers induction in cultivars treated with γ-radiation initiated 5 days earlier than in the non-irradiated control (Li *et al*., 2005). Our results are in conformity with the results reported by Li *et al*. (2005). The results are also in accordance with the findings of Hoque (2010).

Number of microtuber per tube

The analysis of variance for number of microtuber per tube showed highly significant results for radiation doses and interaction between varieties and radiation doses. The varieties showed highly significant results singly as shown in Table 1. It was observed that Diamant produced higher average number of microtuber per tube (0.70) followed by Cardinal and Desiree (0.70, 0.67 respectively) (Table 2). The difference between all three varieties means was non-significant. Table 2 shows the comparison of means of gamma radiation doses. It is clearly visible that treatment 3 Gy Gamma radiations induced maximum number of microtubers (0.92) per tube followed by 5Gy radiation (0.78). Gamma radiation 1 Gy, 0 Gy and 7 Gy produced 0.66, 0.56 and 0.51 number of microtubers per tube, respectively. All treatment means are significantly different with one another, except T1 and T5 which were statistically similar with each other. Interaction between varieties and radiation doses (Table 1) showed that variety Diamant developed maximum (0.98) number of microtubers per test tube with 3 Gy Gamma rays dose followed by variety Cardinal and Desiree, which produced 0.96 and 0.81 microtubers per plant, respectively. The microtuber numbers of variety Diamant and Cardinal were statistically at par on 3 Gy gamma rays. The interaction also showed that the highest dose of Gamma radiation (7 Gy) produced lowest number of microtuber per test tube in variety Cardinal and Diamant but in Desiree the lowest number of microtuber per test tube was produced at 0 Gy gamma rays.

Our results are similar with the results of Al-Safadi *et al.* (2000) who found that irradiation of the explants with 2.5 Gy of gamma radiation led to a significant increase in the number of microtubers (38% increases over the control).

Weight of microtuber (mg)

The analysis of variance for microtuber weight (Table 1) showed highly significant results for radiation doses, varieties and interaction between varieties and radiation doses.

The comparison of means for different doses of gamma radiation is shown in Table 3 It is clear that the highest (73.05 mg) microtuber weight was produced by 3 Gy Gamma radiations dose followed by 5 Gy radiations which was the next best with 65.99 mg microtuber weight. Gamma radiation 1 Gy, 0 Gy and 7 Gy produced 63.03 mg, 60.24 mg and 55.88 mg weight of microtubers, respectively. All treatment means were significantly different with one another, except T1, T2 and T4 which were statistically at par with each other. It was noted that Diamant produced maximum microtuber weight (69.84 mg) followed by Cardinal and Desiree (65.82 mg and 55.26 mg respectively) (Table 2). All three varieties mean were significantly different from one another. The Table 1 also shows interaction between varieties and radiation doses.

Table 1: Analysis of Variance of Shoot length, Number of leaves, Number of Nodes, Root Number, micro tuber formation, micro tuber per tube, microtuber weight, microtuber length , Vit c in potato and starch of microtuber

Source of variation	Df	SL	NL	NN	NR	MTF	MT/P	MTW	MTL	VitC C	SMT%
R. Dose	4	5.95919**	13.0616**	24.48**	85.53**	343.017**	0.328**	498.29**	1.632**	13.589**	28.958**
Varieties	2	1.98882**	0.7162**	4.47**	0.17**	118.154**	0.008**	1133.35**	4.120**	14.948**	5.066**
R Doses x Varieties	8	1.23418**	1.7599**	2.35**	0.17**	39.404**	0.022**	213.68**	0.447**	0.307**	0.483**
Error	45	0.15056	0.1966	0.31	0.042	3.010	0.007	14.55	0.057	1.450	1.122**

NS = Non-significant (P>0.05); * = Significant (P<0.05); ** = Highly significant (P<0.01); **SL**: Shoot length; NL: Number of leaves; NN: Number of Nodes; NR: Root Number; MTF: micro tuber formation; MT/M: micro tuber per tube; MTW: microtuber weight; MTL: microtuber length; VitC C: Vitamin C; SMT%: Starch of micro tuber

Table 2: Effect of varieties on Shoot length, Number of leaves, Number of Nodes, Root Number, micro tuber formation, micro tuber per tube, microtuber weight, microtuber length , Vit c in potato and starch of microtuber

Variety	SL	NL	NN	NR	MTF	MT/P	MTW	MTL	VitC C	SMT%
Diamant	**3.91 A**	**4.06 B**	**6.21 B**	**1.25 A**	**18.15 C**	**0.7A**	**69.84 A**	**4.14 A**	**12.11 B**	**14.30 A**
Desiree	**3.29 B**	**4.43 A**	**6.86 A**	**1.08 B**	**22.85 A**	**0.66B**	**55.26 C**	**3.37 B**	**13.16 A**	**13.90 AB**
Cardinal	**3.68 A**	**4.33 AB**	**5.93 B**	**1.23 A**	**21.57 B**	**0.7A**	**65.82 B**	**3.35 B**	**11.442 B**	**13.30 B**

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean. SL: Shoot length; NL: Number of leaves; NN: Number of Nodes; NR: Root Number; MTF: micro tuber formation; MT/M: micro tuber per tube; MTW: microtuber weight; MTL: microtuber length; VitC C: Vitamin C; SMT%: Starch of micro tuber

Table 3: Effect of gamma radiation dose on Shoot length, Number of leaves, Number of Nodes, Root Number, micro tuber formation, micro tuber per tube, microtuber weight, microtuber length , Vit c in potato and starch of microtuber

Treatment	SL	NL	NN	NR	MTF	MT/P	MTW	MTL	VitC C	SMT%
0 Gy T1	4.68 A	5.87 A	8.75 A	5.97 A	23.75 B	0.57 D	60.24 C	3.34 C	10.89 C	12.50 D
1 Gy T2	4.02 B	4.73 B	6.20 B	0.00 B	16.04 D	0.66 C	63.03 BC	3.64 B	11.83 BC	13.67 C
3 Gy T3	3.26 C	3.90 C	6.09 B	0.00 B	14.91 D	0.92 A	73.05 A	4.12 A	12.66 B	15.92 A
5 Gy T4	3.10 C	3.60 CD	5.60 C	0.00 B	21.91 C	0.78 B	65.99 B	3.79 B	13.74 A	14.83 B
7 Gy T5	3.06 C	3.27 D	5.03 D	0.00 B	27.66 A	0.51 D	55.89 D	3.20 C	12.08 B	12.25 D

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05). Small letters represent comparison among interaction means and capital letters are used for overall mean. SL: Shoot length; NL: Number of leaves; NN: Number of Nodes; NR: Root Number; MTF: micro tuber formation; MT/M: micro tuber per tube; MTW: microtuber weight; MTL: microtuber length; VitC C: Vitamin C; SMT%: Starch of micro tuber

Variety Diamant yielded the highest (82.47 mg) weight of microtuber with 3 Gy Gamma rays dose followed by variety Cardinal which produced 79.21 mg microtuber weight. But the variety Desiree produced maximum (60.03 mg) microtuber weight at 5 Gy dose. The microtuber weight of variety Diamant and Cardinal were statistically similar at 3 Gy gamma rays. It further show that the highest dose of Gamma radiation (7 Gy) produced lowest weight of microtuber. (Table 3). These results were also confirmed by Wiendl *et al.* (1995) who found that with the application of gamma rays the onion production increased by increasing in the bulb weight.

Diameter of microtuber (mm)

The analysis of variance for diameter of microtuber showed highly significant results for radiation doses and varieties (Table 1 and 3). It was observed that Diamant produced maximum microtuber diameter (4.14 mm) as compared to Desiree and Cardinal which possessed 3.37 mm and 3.35 mm diameter of microtuber respectively (Table 2). All variety means were significantly different from one another. Table 3 shows the comparison of means for different doses of gamma radiation used. It is clearly visible that 3 Gy Gamma radiations dose generated the highest (4.12 mm) microtuber diameter than other radiation doses. 5 Gy, 1 Gy, 0 Gy and 7 Gy gamma radiation doses ranked 2nd (3.97 mm), 3rd (3.64), 4th (3.34 mm) and 5th (3.20 mm), respectively for diameter of microtuber as shown in Figure 2. The data also shows that 1 Gy and 5 Gy treatments were statistically similar with each other. Similarly, 0 Gy and 7 Gy treatments were also statistically similar with each other. The interaction between varieties and radiation doses is shown in Table 1. Variety Diamant developed the highest (4.96 mm) diameter of microtuber with 3 Gy Gamma radiation dose followed by variety Cardinal and Desiree which produced 3.83 mm and 3.59 mm diameter of microtuber respectively as shown in Figure 3. The interaction also showed that the highest dose of Gamma radiation (7 Gy) produced the lowest weight of microtuber in varieties Cardinal and Desiree, but in Diamant the lowest microtuber weight was produced on control treatment.

Our results are in line with the findings of Bajaj (1970) who reported an increase of tissue culture growth of bean *(Phaseolus vulgaris)* following irradiation.

Figure 2: Microtuber formation in variety Cardinal treated with gamma rays (1, 3, 5, 7 Gy) after 8 weeks of nodal culture irradiation.

Vitamin C content of fresh microtubers (mg/g)

Analysis of variance for vitamin C content of fresh microtubers showed highly significant results for radiation doses and varieties (Table 1). The effect of varieties radiation doses was significant. The cultivar Desiree produced significantly the highest Vitamin C contents (13.16 mg/g) as compared to other two varieties. Diamant and Cardinal were statistically non significant with each other and produced 12.11 mg/g and 11.44 mg/g Vitamin C contents, respectively (Table 2). Gamma radiation doses significantly affected the Vitamin C contents. 5 Gy treatment produced the highest contents of vitamin C (13.74 mg/g) followed by 3 Gy, 7 Gy, 1 Gy and 0 Gy treatments which produced 12.08 mg/g, 12.66 mg/g, 11.83 mg/g and 10.86 mg/g vitamin C contents, respectively. All treatments are statistically non significant with each other except 5 Gy treatment which was different from other treatments (Table 3).

Table 1 shows the interaction between varieties and radiation doses. Variety Desiree produced the highest (14.86 mg/g) vitamin C contents of microtuber with 5 Gy Gamma radiation dose followed by variety Diamant and Cardinal which produced 13.40 mg/g and 12.97 mg/g vitamin C content of microtubers, respectively. At control all three varieties produced the lowest amount of vitamin C contents.

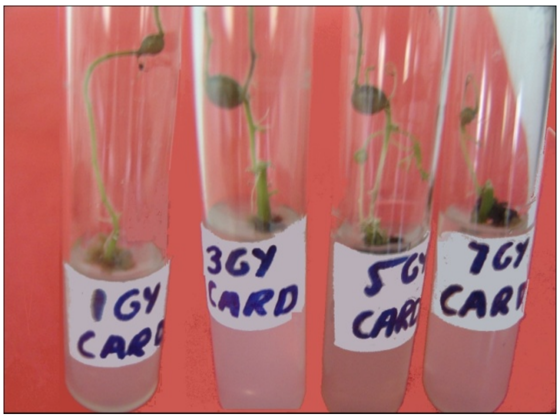

Figure 3: Microtuber formation in three varieties (Cardinal, Desiree and Diamant) treated with gamma rays (3 Gy) after 8 weeks of nodal culture irradiation

Our results are confirmed by Li *et al*. (2005) who stated that high doses (6 - 8 Gy) enhanced ascorbic acid in potato.

Starch of dried Microtubers (%)

Analysis of variance for starch contents of dried microtubers showed highly significant results for radiation doses and significant results for varieties (Table 1). The effect of varieties and radiation doses was significant. The cultivar Diamant produced significantly higher starch contents (14.30 %) which was statistically at par with Diamant and Desiree which produced 13.90 % and 13.30 % starch contents, respectively (Table 2). Gamma radiation doses significantly affected the starch contents (Table 3). Treatment 3 Gy produced the highest contents of starch (15.92 %) followed by 5 Gy, 1 Gy, 0 gy and 7 Gy treatments which produced 14.83 %, 13.67%, 12.50% and 12.25% starch contents, respectively. All treatments were statistically significant with each other except 0 Gy and 5 Gy treatments which were similar with each other. Table 1 shows the interaction between varieties and radiation doses. Variety Diamant produced the highest (16.50 %) starch contents in microtuber with 3 Gy Gamma radiation dose followed by variety Desiree and Cardinal which produced 15.75 % and 15.50 % starch content in microtubers, respectively. On control all three varieties produced the lowest amount of starch contents which were at par with 7 Gy radiation dose.

Our results are in accordance with the results of Li *et al*. (2005) who stated that low dose irradiation (2 - 4 Gy) increased the starch content of microtubers.

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