

EFFECT OF GAMMA RAYS ON CALLUS GROWTH AND PLANTLET REGENERATION IN GARLIC

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ABSTRACT

This study was carried out in the Tissue Culture Laboratory, Horticulture Department, Faculty of Agriculture, Kafr El-Sheikh University in 2014 for increasing genetic variability plants. Fresh callus (21 days old) derived from root tips segments from Balady cv. was exposed to nine doses of gamma rays (0, 50, 100, 150, 200, 250, 500, 1000 and 2000 rad). After 21 days from radiation treatments, low doses of gamma rays stimulated callus growth, while higher doses decreased them. Low doses of gamma rays (50 rad) produced the highest callus fresh weight, while, 2000 rad produced the lowest value. After 60 days from radiation treatments, the effect of radiation was continuously noticed. Callus fresh weight was increased with increasing the radiation dose from 50 to 100 rad, and then decreased with increasing gamma irradiation doses. Therefore the lowest value of callus fresh weight was recorded at 2000 rad. Gamma rays at 100 rad produced the lowest differentiated plantlets per callus (normal, abnormal and total). Callus produced 100% normal plantlets at the low doses of radiation, i.e., from 50 to 250 rad, however some of abnormal plantlets were produced at the higher doses of gamma rays. Percentage of abnormal plantlets increased by increasing gamma ray doses until 500 rad which produced the highest record (18.1%).

INTRODUCTION

Garlic (*Allium sativum* L.), is an important widely cultivated crop, which it used for both culinary and medicinal purposes. In Egypt, garlic is considered one of the most important vegetable crops. Although, garlic production in Egypt is considered the highest proportion per unit area in the world, but garlic production (Balady cv.) has been deteriorated in their characters. This is due to using this cultivar in agriculture since the Pharaohs time. The cultivated genotypes of garlic are sexually sterile (Barandiaran *et al.* 1999). Accordingly, improvement of garlic crop is very limited. Therefore, tissue culture techniques may be very useful for breeding programs. Moreover, radiation treatments may increase the somaclonal variation and it was possible to select useful altered plants *in vitro* (Suciu *et al.*, 1991; Croci *et al.*, 1990; Al-Safadi *et al.*, 2002). The present investigation aimed to induce maximum variation in garlic plants through callus irradiation to increase genetic variability in garlic plants derived from tissue culture technique to select the suitable one (s).

MATERIALS AND METHODS

This study was carried out at the Tissue Culture Laboratory, Horticulture Department, Faculty of Agriculture, Kafr El-Sheikh University in 2014. Balady cv. of garlic (*Allium sativum* L.) was used in this study.

Callus induction and irradiation treatments:

Root segments with 0.8 to 1.2 cm long containing the apical meristem excluding the terminal quarter of its end were cultured on induction media Murashige-Skoog (MS) medium (Murashige and Skoog, 1962) supplemented with 1.1 mg/L 2,4-Dichlorophenoxyacetic acid (2,4-D) + 2.0 mg/L Kinetin (Kin) + 1.7 mg/L indole-3-acetic acid (IAA) (Metwally *et al.*, 2012). Fresh callus (21 days old) derived from root tip segments from Balady cv. was exposed to nine doses of gamma rays; i.e., 0, 50, 100, 150, 200, 250, 500, 1000 and 2000 rad. in the Cesium Gamma Cell, National Center for Radiation Research and Technology, Nasr City, Cairo. (GC40 model, type of source: ¹³⁷Cs, dose rate: 0.996 rad/second, irradiation chamber was 40 cm diameter and 10 cm height. After one day from the irradiation treatments, the irradiated calli were transferred to the same fresh induction media. Afterward, the materials were incubated in the dark in the growth chamber at a temperature of 26 °C ± 2, controlled automatically. The tested treatments were arranged in a randomized complete design. Data were collected from four petri dishes as a replicate, each dish contained 5 calli. After 21 and 60 days the following data were recorded: Callus fresh weight (mg) and changing rate in fresh weight percentage compared with control.

Plantlets regeneration

After two months from the radiation treatments, irradiated calli were transferred to differentiation medium which consists of MS medium supplemented with 2.0 mg/L 6-benzyladenine (BA) + 1.0 mg/L 1-naphthalene acetic acid (NAA) (Metwally *et al.*, 2012) in dishes (7.5 cm) for four weeks then, in jars 375 ml. Calli were incubated at 26 °C ± 2, 16 hours light/day and light intensity 2200-2400 Lux. After four months from incubating the radiated calli on the differentiation media, the following data were recorded: Number of normal, abnormal and total plantlets per callus and percentage of normal and abnormal plantlets per callus. The obtained data were subjected to statistical analysis as randomized complete block design with four replicates according to Snedecor and Cochran (1972). Means of treatments were compared using Duncan's multiple range test at 0.01 probability according to Duncan (1955).

RESULTS AND DISCUSSION

1. Callus characters

Callus fresh weight (mg)

Data in Table (1) and Figs. (1 & 2) show the effect of different doses of gamma rays on callus fresh weight for Balady cv. after 21 days of radiation treatments. In this respect, significant differences among different doses of gamma rays were recorded. Low doses of gamma rays (50 rad) produced the heaviest callus fresh weight, while, the highest dose of gamma rays (2000

rad) produced the lightest ones. After 60 days from radiation treatments data in Table (1) and Fig. (1) show the effect of radiation remained as previously mentioned with increasing the differences. Callus fresh weight increased as the dose increased from 50 to 100 rad, and then such weight decreased with increasing gamma irradiation doses. The lowest value of callus fresh weight was recorded at 2000 rad of gamma rays. The effect of radiation on callus fresh weight appeared with the high doses after 21 days from radiation treatments while, this effect appeared with all doses after 60 days of the radiation.

The stimulatory effect of the low doses of gamma rays on growth of callus can be attributed to the increase in auxin concentration as a result of ionizing radiation. While, the higher doses may lead to dropping off in auxin concentration in metabolically active tissues and hence led to a reduction in the growth of callus. This drawn conclusion goes along with the results of Croci *et al.* (1990). While, Al-Safadi and Simon (1990) found that, 500-1000 rad increased callus fresh weight of carrot. Also on sugarcane, Singh and Singh (1993) reported that, 1000 rad increased callus dry weight, but 2000 rad decreased it.

Change rate in callus fresh weight compared to control

Data in Table (1) illustrate that, after 21 days of radiation treatments, no significant differences were noticed among low doses of gamma rays, i.e., 50, 100, 150 and 200 rad. While, increasing the dose of gamma rays from 250 to 2000 rad caused the bigger negative changing rate in callus fresh weight compared with the control as 2000 rad of gamma rays recorded - 451% compared to control.

Table (1): Callus fresh weight and changing rate in fresh weight percentage (compared with control) as affected by gamma rays doses on Balady cv.after 21 and 60 days from radiation treatments during 2000 / 2001 season.

Gamma rays dose (rad).	Callus fresh weight (mg)				Changing rate in callus fresh weight percentage Compared with control			
	After 21 days from radiation treatments		After 60 days from radiation treatments		After 21 days from radiation treatments		After 60 days from radiation treatments	
0	173.0	a	697.0	c	0.0	a	0.0	c
50	183.0	a	701.0	b	40.0	a	16.0	b
100	164.2	a	709.0	a	-35.0	a	48.0	a
150	168.4	a	524.0	e	-18.0	a	-692.0	e
200	172.8	a	531.0	d	-1.0	a	-664.0	d
250	127.4	b	316.0	g	-181.0	b	-1524.0	g
500	102.6	c	389.0	f	-282.0	c	-1232.0	f
1000	84.8	c	301.0	h	-353.0	c	-1584.0	h
2000	60.2	d	160.0	i	-451.0	d	-2148.0	i
F test	**		**		**		**	

** significant at 0.01 level of probability.

Means having the same letters don't significantly differ at 0.05 level of probability according to Duncan's multiple range test

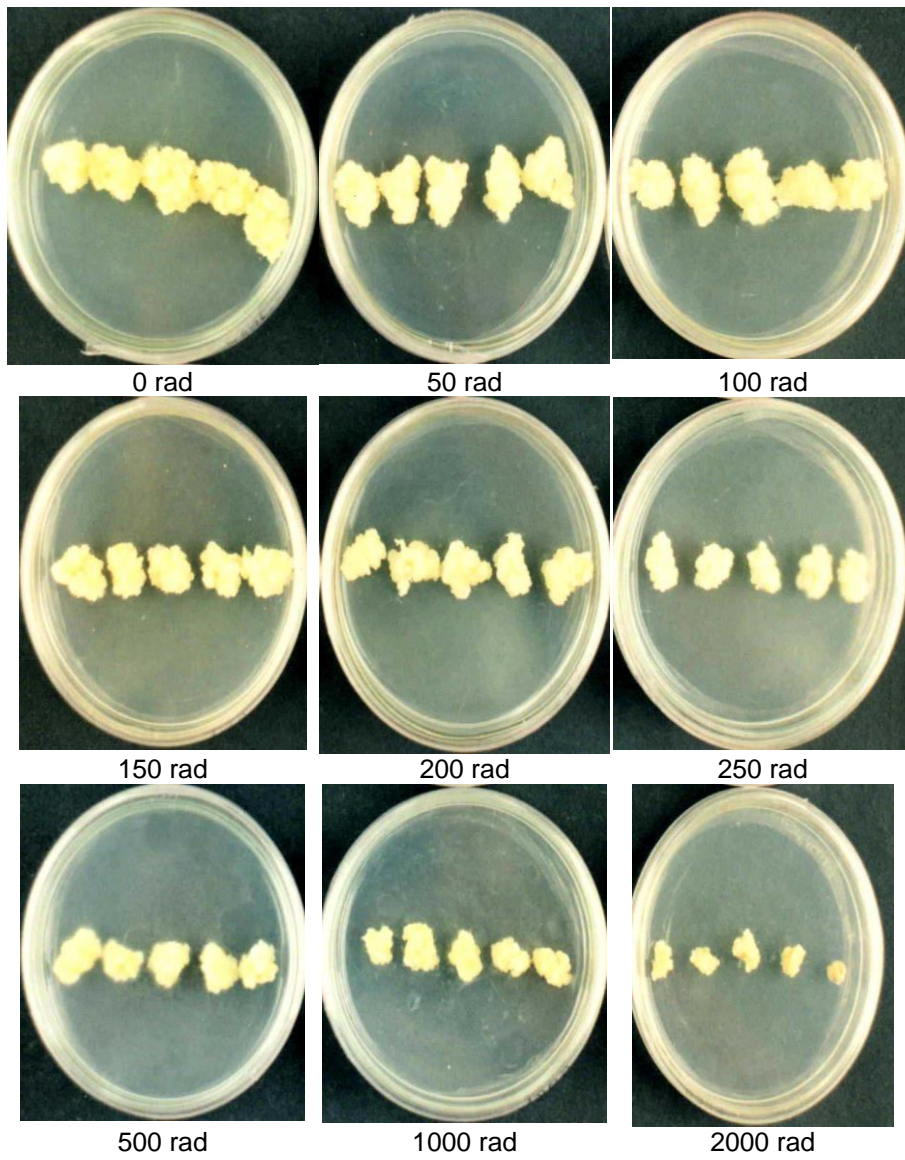


Fig.1. Effect of different doses of gamma rays on callus growth after 60 days from radiation treatment.

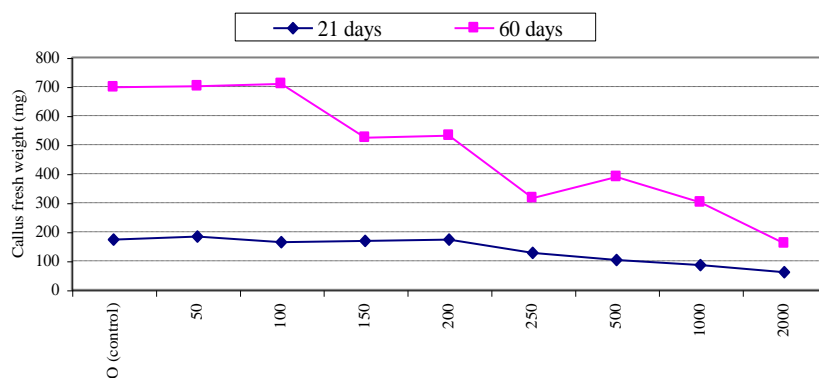


Fig. (2): Callus fresh weight (mg) as affected by radiation doses on Balady cv. calli after 21 and 60 days from radiation treatments.

After 60 days from radiation treatment, data showed that, there were significant differences among gamma rays doses as it affected changing rate in callus fresh weight compared to the control. Low doses of gamma rays increased changing rate of callus weight compared with the control, then the rate was decreased with increasing gamma doses from 150 rad till 500 rad. Continuous decrease changing rate of callus weight was noticed due to increasing gamma rays, as 2000 rad caused – 2148% changing rate comparing to control.

The high doses of radiation caused a reduction in the growth regulator, which was detriment to the mitotic activity of the callus consequently, suppressed growth, i.e., callus fresh weight. This drawn conclusion was confirmed by the results of Abdel-Maksoud (1992).

2. Plantlets regeneration

Number of normal plantlets per callus

Data presented in Table (2) and Fig. (3) show that, using gamma irradiation at a dose of 2000 rad produced the greatest number of normal plantlets per callus, while, 100 rad produced the lowest number. Generally, low doses, i.e., 50, 100 and 150 rad. produced the lowest number of normal plantlets per callus, while the higher doses, i.e., 1000 and 2000 rad produced the greatest one.

This result may be explained by the fact that high doses of gamma rays induced mutations and increased shoots differentiation. These results are in agreement with those of Singh and Singh (1993), who found that, exposure of sugarcane callus to 1000 or 2000 rad gamma rays regenerated more plantlets than the non-irradiated.

Generally, when irradiated callus was transferred to regeneration medium, plantlet formation was inhibited for 4 weeks compared with control. These results agree with those of Zehn (1997) on callus of garlic.

Number of abnormal plantlets per callus

As for number of abnormal plantlets per callus as affected by different doses of gamma rays, significant differences were recorded in this respect

(Table 2 and Fig. 3). Callus of Balady cv. did not produce any abnormal plantlets when treated by gamma rays until 250 rad. These results mean that, Balady cv. was resistant to the damage effect of gamma rays until 250 rad. Afterwards, increasing gamma ray doses from 250 rad to 500 rad produced more damage, which 7.5 abnormal plantlets per callus was recorded. After increasing doses of gamma rays to 1000 and 2000 rad the abnormal plantlets recorded 4.0 and 5.4 per callus, respectively.

These results were confirmed by those of Al-Safadi and Simon (1990), who found that, the frequency of abnormal plants, i.e., albinos, plants with no roots or deformation of leaves regenerated from carrot cell culture were increased by gamma irradiation.

Total number of plantlets per callus

Table (2) and Fig. (3) declare that, there were significant differences in total number of plantlets per callus as affected by different doses of gamma rays. Using 1000 and 2000 rad of gamma rays achieved the greatest number of total plantlet number per callus after 90 days from sowing on differentiation medium. While, the lowest number was obtained from 100 rad of gamma rays. Generally, 100 rad of gamma rays produced the lowest differentiated plantlets per callus (normal, abnormal and total). While, the above mentioned dose produced the biggest callus. This means that, 100 rad dose of gamma rays may increase the growth regulators that exerted a positive effect on callus growth, but did not induce the balance in plantlets regeneration. Also, it may be due to the increase in cell division by 100 rad of gamma rays compared with other gamma ray doses.

Table 2 Number of normal, abnormal, total plantlets per callus, percentage of normal/total plantlets number per callus and percentage of abnormal/total plantlets number per callus as affected by gamma rays doses after 90 days on differentiation medium during 2000/01 season.

Gamma rays (rad)	No. of plantlets / callus						Normal/total plantlets/ callus (%)		abnormal/total plantlets/ callus (%)	
	Normal		Abnormal		Total					
0 (control)	36.3	bc	0.0	c	36.3	bc	100.0	a	0.0	c
50	23.5	de	0.0	c	23.5	d-e	100.0	a	0.0	c
100	22.0	e	0.0	c	22.0	e	100.0	a	0.0	c
150	28.0	c-e	0.0	c	28.0	d-e	100.0	a	0.0	c
200	38.5	b	0.0	c	38.3	bc	100.0	a	0.0	c
250	30.8	b-d	0.0	c	30.8	cd	100.0	a	0.0	c
500	33.0	b-c	7.5	a	40.5	b	81.9	c	18.1	a
1000	49.0	a	4.0	b	53.0	a	92.3	b	7.7	b
2000	53.5	a	5.4	ab	58.3	a	91.9	b	8.1	b
Mean	34.9		1.9		36.7		96.2		3.8	

All characters showed significant differences at 1% level **.

Means followed by a common letter are not significantly different at the 5 % level according to Duncan's multiple range test.

The obtained results are in agreement with those reported by Jain *et al.* (1984), who found that, shoot regeneration from *Datura innoxia* callus was

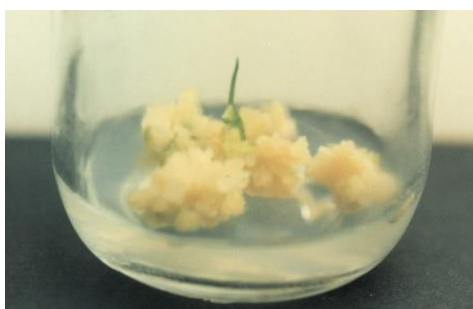
stimulated by the 200 and 1000 rad treatments. Al-Safadi and Simon (1990) on carrot recorded that, gamma rays stimulated shoot formation at 1000 rad, and inhibited it at the higher doses. While, Zehn (1998) found that, the number of globular bodies (were capable of differentiating into plantlets) of leaf callus of garlic decreased at higher radiation doses (1000 rad).

Percentage of normal plantlets per callus

The callus produced 100% normal plantlets at the low radiation doses, i.e., 50 to 250 rad. and produced some of abnormal ones at the higher doses of gamma rays. The lowest percentage of normal plantlets was obtained from 500 rad of gamma rays (Table 2).

Percentage of abnormal plantlets per callus

Concerning the effect of different doses of gamma rays on abnormal plantlets percentage per callus, Table (2) declare that, significant differences among different doses of radiation were noticed. Percentage of abnormal plantlets increased by increasing gamma ray doses, as the higher dose (500 rad) produced the highest record (18.1%). While low doses of radiation (50 to 250 rad) did not produce abnormal plantlets from callus.



a. Starting of plantlets regeneration after four weeks on differentiation medium

b. Plantlets before rooting



c. Development of root formation.

Fig. 3. Development of plantlets differentiation from callus.

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تأثير أشعة جاما على نمو الكالس وتميز النباتات في الثوم
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أجريت هذه الدراسة في معمل زراعة الأنسجة بقسم البساتين ، بكلية الزراعة ، جامعة كفر الشيخ ، خلال موسم ٢٠١٤م. وتهدف هذه الدراسة إلى زيادة التباين الوراثي بين نباتات الثوم وذلك بتشجيع الكالس بتسع جرعات من أشعة جاما وهى صفر ، ١٠٠٠ ، ٥٠٠ ، ٢٥٠ ، ٢٠٠ ، ١٥٠ ، ١٠٠ ، ٥٠ و ٢٠٠٠ راد سجلت اختلافات معنوية في نمو الكالس و صفاته نتيجة المعاملة بجرعات مختلفة من أشعة جاما. فبعد ٢١ يوم من تشجيع الكالس أعطت الجرعات المنخفضة (٥٠ راد) أعلى وزن طازج للكالس ، بينما سجلت الجرعة المرتفعة (٢٠٠٠ راد) أقل وزن للكالس. كذلك ، و بعد ٦٠ يوما من التشجيع ، زادت الاختلافات بين الجرعات و زاد وزن الكالس مع الجرعات ٥٠ و ١٠٠ راد فقط عن الكنترول ، ثم انخفض الوزن مع زيادة جرعة الأشعة حيث سجلت أقل قيمة لوزن الكالس مع الجرعة ٢٠٠٠ راد. و بصفة عامة ، فإن الجرعات المنخفضة من أشعة جاما نشطت نمو الكالس ، بينما الجرعات المرتفعة خفضت من النمو. أيضا تأثير الإشعاع على الكالس كان أكبر بعد ٦٠ يوما من التشجيع مقارنة بتأثيره بعد ٢١ يوما من التشجيع. اختلف عدد النباتات الناتجة من كل كالس (عدد كلي ، عدد طبيعي أو عدد مشوه) باختلاف جرعة أشعة جاما ، و كانت الاختلافات معنوية. فقد أعطت الجرعات المنخفضة من أشعة جاما (٥٠ إلى ٢٥٠ راد) نباتات ذات نمو طبيعي ، بينما أعطت الجرعة ١٠٠ راد أقل عدد من النباتات المتميزة لكل كالس. زاد عدد النباتات المشوهة بزيادة جرعة أشعة جاما ، فقد أدى استخدام أشعة جاما بجرعة قدرها ٥٠٠ راد إلى إنتاج أقل نسبة للنباتات السليمة و أعلى نسبة للنباتات المشوهة (١٨.١%).