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إنتاج طفرات من الثوم عن طريق المعاملة بأشعة جاما وزراعة الكالس

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الملخص العربى

أجريت هذه الدراسة بهدف إنتاج تباينت وراثية فى الثوم (الصنف البلدى) عن طريق تعريض الفصوص أو الكالس إلى جرعات مختلفة من أشعة جاما (صفر ، ٢٥ ،٥ ، ٧٥ ، ١٠ جراى). اوضحت النتائج أن تعريض الكالس الى ٥ جراى أدى إلى إنتاج أكبر عدد من النباتات لكل كالس ، بينما أعطت معاملة الكنترول أقل عدد من النباتات لكل كالس. كما أعطت معاملة الفصوص ب ٧٥ جراى الى الحصول على أعلى عدد من النباتات لكل كالس.

تم الحصول على أعلى نسبة مئوية للنباتات غير الطبيعية والتباينات السيتوبلازمية عندما تم تعريض الكالس والفصوص الى ٥ جراى. وقد تم ايضا دراسة تحليل الهجرة الكهربائية لمشابهات الانزيمات لكل من إنزيمى االستريز والبيروكسيديز للنبتات الناتجة من معاملة الكالس والفصوص بأشعة جاما

IN VITRO INDUCTION OF MUTATION IN GARLIC THROUGH GAMMA RADIATION AND CALLUS CULTURE

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ABSTRACT: *This study reports on inducing genetic variability in the Egyptian garlic (Balady cv.) by exposing garlic cloves and calluses to gamma irradiation at doses of 0, 2.5, 5, 7.5 and 10 Gy. Irradiated calluses at 5 Gy gave the highest number of plantlets per callus, whereas the unirradiated treatment (0Gy) gave the lowest number of plantlets per callus. Similar result was observed in cloves which treated with 7.5 Gy. The higher percentages of abnormal plantlets with cytological irregularities were obtained from irradiated calluses and cloves at 5 Gy. Electrophoretic analysis for both esterase and peroxidase isozymes of the regenerated plants produced from callus and cloves irradiation was studied.*

Key wards: Abnormalities, *Allium sativum*, esterase, irradiation, mutation, peroxidase,

INTRODUCTION

Garlic (*Allium sativum* L.) is a widely consumed vegetable crop, which is known for its flavor and medicinal properties (Nagovrney, 1998). Recently, its therapeutic value has been related to cardiovascular diseases, cholesterol metabolism, atherosclerosis (Collin, 2004) and cancer (Le Bon and Siess, 2000). Therefore, garlic products became very popular in the markets and many pharmaceutical garlic preparations are now available as traditional medicines.

Garlic is an obligate apomictic plant which is vegetatively propagated by cloves (Novak *et al*., 1986) except for Tunceli garlic which produces flowers (Ipeka et al. 2008). Therefore, the multiplication rate is low and the improvement of this crop is limited. Also, due to difficulties of inducing flowers, breeding programs have been limited to clonal selection and production of disease-free stocks. There are few reports on improved garlic by clonal selection (Koul *et al*., 1979 and Ayabe *et al.*1995). However, the progress in classical breeding methods of garlic is restricted. Somaclonal variations obtained through plant cell and tissue culture offer an opportunity to broaden the genetic variability of crops. From the breeding point of view, single gene mutations are the most important mutants since they allow small but significant positive changes resulting in desirable traits such as stress tolerance or disease resistance (Bozorgipour and Snape, 1997 and Yan *et al*., 2009). Moreover, mutation may increase the genetic variability and it has proved the possibility to select mutant of breeding interest *in vitro* (Manjula *et al.,* 2000). Gamma irradiation increased variability of garlic plants with respect to some characters, such as bulb weight, clove weight and date of maturity (El-Denary, 2004).The objective of the present study was to induce genetic variability in Egyptian garlic (Balady cv.) through gamma irradiation and tissue culture techniques with the aim to improve its economic value.

MATERIALS AND METHODS

This study included two experiments, which were carried out during 2009 and

2010 at the Horticulture Department, Faculty of Agriculture, Kafrelsheikh University.

1. Media preparation

MS basal salts (Murashige and Skoog, 1962) and B5 vitamin (Gamborg *et al.* 1968) were used. All media were supplemented with sucrose at 30 g l^{-1} and activated charcoal at 0.5 g l^{-1} and solidified with 8 g l^{-1} agar. The pH of all media was adjusted to 5.7 before autoclaving at 121ºC for 15 minutes under a pressure of 1.1 $kgcm^{-2}$.

2. Preparation of garlic cloves (Balady cv.)

The outer cover of bulb was removed and the cloves were surface sterilized using 70% (*v/v*) ethanol for 30 sec. followed by 50% (v/v) NaOCl (commercial bleach Clorox having 5.25% sodium hypochlorite) with few drops of Tween 20 for 20 min. Then, they were rinsed 3 times with sterile distilled water. Finally, they were cultured in glass jars containing 35 ml of half strength MS salts as an emergence medium. The cultures were incubated in a growth chamber at 27 ± 2 ºC for 3 days under dark then, kept under a photoperiod of 16/8 h (light/dark) at light intensity at 30-33 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) until germination.

3. Callus induction and irradiation treatments

In the first experiment, root segments (0.8 – 1.2 cm) obtained from *in vitro* cultured cloves were used for callus induction. Root segments were vertically cultured in Petri dishes containing 15 ml of MS medium supplemented with 1.0 mg l^{-1} 2,4-D (2,4 dichlorophenoxy acetic acid). Each Petri dish contained five root segments. The callus induction started after 15 days from root culture. Calluses (21 days old) derived from root tips were exposed to different doses of gamma irradiation at 0, 2.5, 5, 7.5 and 10 Gy using Ceasium Gamma Cell (GC 40 model, type of source: 137 CS, dose rate: 0.0996 rad/sec, irradiation chamber was 40 cm diameter and 10 cm height), National Center for Radiation Research and Technology, Nasr City, Cairo.

The irradiated calluses were transferred to fresh medium after four hours from the irradiation treatments. Then, the cultures were incubated in a growth chamber at 26 ± 2 ºC under dark conditions. After 40 and 70 days from irradiation treatments, the callus fresh weight (mg) and callus diameter (mm) were measured.

For the second experiment, garlic bulbs were randomly chosen and classified into five groups each of which weighted 250 g of cloves. These groups were exposed to the five doses of gamma rays (0, 2.5, 5, 7.5 and 10 Gy) and then cultured following the same procedures described in the first experiment.

4. Plantlets regeneration

Ninety-days old calluses were transferred into differentiation medium (MS salts plus B5 vitamin supplemented with 2 mg l^{-1} BA and 1 mg l^{-1} NAA) in sterile Petri dishes for four wks. Calluses were incubated at 26 ± 2 ºC, 16/8 hours light/dark and light intensity of 30-33 PPFD. The regenerated adventitious shoots appeared after 3 months on the differentiation media and the plantlets regeneration increased with callus sub-culturing at four weeks intervals. After 3 months the number of embryos was recorded. In addition, after four months the following data were recorded: number of normal plantlets per callus, number of abnormal plantlets per callus, total number of plantlets per callus, percentages of normal plantlets and abnormal plantlets per callus. Abnormal plantlets included albinos, rootless plants or deformed leaf shape, color and/or size stem color were calculated according to Al-Safadi and Simon (1990).

5. Experimental design and statistical analysis

The experiments were set up in a completely randomized design. There were 6 replications per each treatment. Each replicate was represented by a Petri dish containing 5 calluses rendering a group of 30 calluses per treatment. Both experiments repeated twice. Data were analyzed by analysis of variance and Duncan's multiple range test was used to test differences between treatment means.

6. Root formation and plant acclimatization

The proliferated clumps of adventitious shoots were individually separated and cultured in jars containing 35 ml of MS medium without plant growth regulators (PGRs) to induce rooting. After four wks, the plantlets were transferred to half strength MS medium without PGRs for root development. Plant acclimatization was carried out according to Ali and Metwally (1992). At the end of the acclimatization, each plant produced a small (5-10 mm diameter) and non-divided bulb (Fig 1) which was considered the first vegetative generation plants.

7. Cytological and isozymes analysis

Root tips of regenerated plantlets were stained using Fuelgen stain as described by Darlington and LaCour (1976) and cytological parameters i.e., mitotic index and chromosomal aberrations were recorded.

The isozyme technique was used as the biochemical analysis method to detect and elucidate some of the genetic variations among regenerated plantlets (second generation) compared to their original cultivar. Two isozymes (esterase and peroxidase) were detected. Staining of the gel was performed as described by Larsen and Benson (1970) for peroxidase and according to Soltis *et al.* (1983) for esterase.

RESULTS AND DISCUSSION

1. Production of mutations through callus irradiation

1.1. Effect of gamma rays on callus induction

Table 1 presents the effect of different doses of gamma rays on callus fresh weight and diameter for garlic, Balady cv. after 40 and 70 days from irradiation treatments. No significant differences were recorded for the different doses of gamma irradiation for callus fresh weight after 40 days, while significant differences were recorded after 70 days from irradiation treatments. The lowest callus fresh weight was recorded with the highest dose (10 Gy) while irradiated callus with lower doses (2.5 and 5 Gy) gave the highest callus fresh weight.

The stimulatory effect of the low doses of gamma rays on callus growth can be attributed to the increase in the endogenous auxin content as a result of ionizing radiation, while the high doses may have caused a reduction in the auxin content in the metabolically active tissues and hence led to a reduction in the callus growth. This drawn conclusion goes along with the results of Croci *et al.* (1990**)**. Similar findings on the effect of gamma radiation on callus growth were reported on carrot (Al-Safadi and Simon, 1990) and garlic (El**-**Denary**,** 2004**)**.

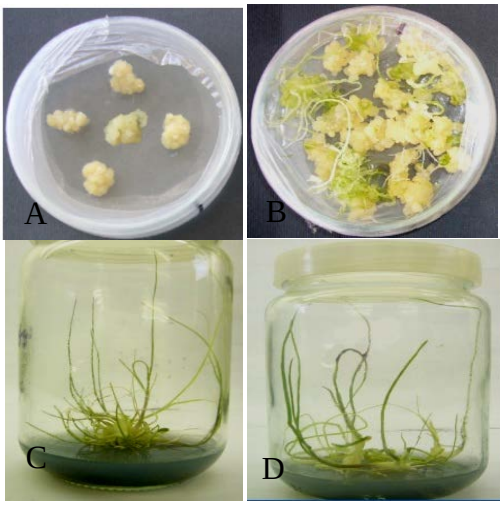

Fig (1): Plantlets regeneration from root cultures of garlic (A: Callus after 90 days from culture B: Plantlet regeneration from callus after 4 weeks on regeneration medium C: Plantlets on rooting medium D: Bulb formation during rooting.

Table (1): Effect of gamma rays on callus induction from root culture of garlic after 40 and 70 days from radiation treatments.

Gamma rays (Gy)	Callus weight (mg)		Callus diameter (mm)	
	40 days	70 days	40 days	70 days
0	143	591ab	10.7 a	12.3 bc
2.5	171	712 a	10.3 ab	14.0 a
5.0	180	692a	9.3 b	14.0 a
7.5	164	593 ab	10.0 ab	13.3 ab
10.0	191	510 b	6.7 c	12.0 c
F. test	N. S.	*	**	*

*, ** and N.S. indicate significant differences at P < 0.05, P < 0.01 and not significant, respectively, according to F. test. Means followed by a common letter in the same column are not significantly different at the 5 % level

All the doses of gamma radiation used significantly reduced the callus diameter compared to that of the control with nonsignificant differences in some cases. The smallest callus diameter was recorded for the highest dose at 10 Gy. Continuous decrease in callus diameter with increasing gamma irradiation doses was reported by El-Denary (2004). The largest callus diameter was obtained from doses of 2.5 and 5 Gy. The inhibition and/or stimulation in callus growth, i.e., diameter could be attributed to the effect of low and high irradiation doses on cell division and cell elongation rate. It was reported that garlic callus growth was accelerated at 1 or 3Gy and was not inhibited at 5 Gy (Zhen, 1997).

1.2. Effect of gamma rays on plantlets regeneration

Table 2 and Fig 2 present the effect of different doses of gamma irradiation on the number of embryos at globular, heart and torpedo stages. The highest number of embryos per callus was recorded for the dose of 5 Gy, however, no significant differences among irradiation doses were recorded. Irradiation at 5 Gy gave the highest number of total plantlets per callus, followed by the dose of either 7.5 or 10 Gy,

while the lowest number was recorded at 0 Gy.

There were highly significant differences among the doses of gamma irradiation for the number of normal plantlets. Radiation dose of 5 Gy produced the highest number of normal plantlets per callus, while 2.5 Gy produced the lowest number of normal plantlets. However, the percentage of normal plantlets at all doses was lower than that of the control. Singh and Singh (1993) reported that exposure of sugarcane callus to 10 Gy gamma irradiation regenerated more plantlets than the non-irradiated one. Cytokinins play an important role in plantlets regeneration from callus of garlic (Zhen, 1997). Irradiation affects the synthesis of cytokinins needed for plantlets regeneration from callus or the uptake of cytokinins from the culture media (Einset and Skoog, 1973).

The highest number of abnormal plantlets was recorded at 5 Gy irradiation dose while the control produced the lowest number of abnormal plantlets. Generally, a wide range of morphological variations were observed in garlic plantlets regenerated from the irradiated calluses. Al-Safadi *et al.* (2002) reported similar results to the present study. They found that garlic leaves grown from irradiated explants had purple to brown spots and their intensity increased with increasing gamma irradiation. Moreover, this could be attributed to the fact that the extreme doses of ionized irradiation induce chromosomal aberrations which could be harmful (Hossain and Alam, 2001). On the other hand, variation in plants regenerated from the non-irradiated callus might be due to the influence of the PGRs added to the culture medium (Vanden Bulk *et al.* 1990).

Table (2): Effect of gamma rays on plantlets regeneration from irradiated callus.

Gamma rays (Gy)	Number of embryos/ callus	Total number of plantlets /callus	Normal plantlets		Abnormal plantlets	
			No./callus	%	No./callus	%
0	13.8	1.60 c	1.3 c	79.1 a	0.30 c	20.9 c
2.5	14.4	2.35c	1.5 c	62.8 c	0.85 c	37.2 a
5.0	17.4	17.2 a	10.2 a	59.3 d	7.0 a	40.7 a
7.5	12.4	8.86 b	6.8 b	76.7 ab	2.10 b	23.3 bc
10.0	14.2	7.86b	5.9 b	74.6 b	1.96 b	25.4 b
F. test	N.S.	**	**	*	**	*

*, ** and N.S. indicate significant differences at $P < 0.05$, $P < 0.01$ and not significant, respectively, according to F. test. Means followed by a common letter in the same column are not significantly different at the 5 % level.

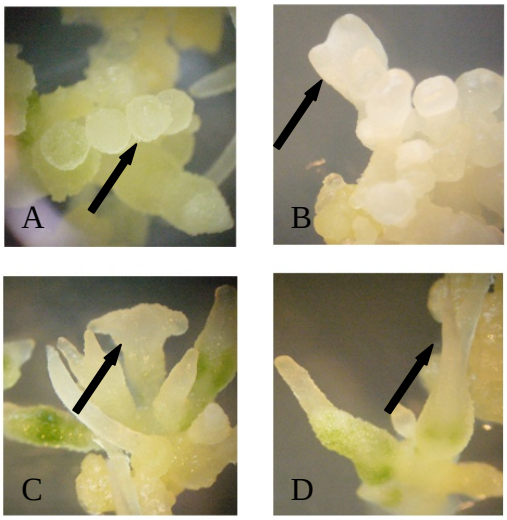

Fig. (2): Different stag culture e (A: Globular embryo B: Heart embryo C: Torpedo embryo D: Plantlet)

2. Production of mutations through cloves irradiation

2.1. Effect of gamma rays on callus induction

Table 3 shows that there are significant differences among different irradiation doses for callus fresh weight after 60 and 90 days of culture on induction medium. Low doses i.e, 2.5 and 5 Gy produced the lowest callus fresh weight, while the control gave the highest one.

For the average callus diameter, all doses produced callus diameters lower than that produced by the control. Slight increase in callus diameter was noticed after the treatment with 7.5 Gy compared to that of 2.5 Gy. However, high doses at 10 Gy reduced the callus diameter.

After 90 days of culture on induction medium, highly significant differences among different doses were recorded for callus weight. The control yielded the highest callus fresh weight and diameter while the lowest callus fresh weight was obtained at doses of 2.5 and 5 Gy. Irradiation at 10 Gy produced the smallest callus diameter.

Table (3): Effect of gamma rays on callus induction from root culture of irradiated cloves after 60 and 90 days from culture.

Gamma rays (Gy)	Callus weight (mg)		Callus diameter (mm)	
	60 days	90 days	60 days	90 days
0	130 a	591 a	10.7 a	12.3 a
2.5	70 b	200 c	6.0 b	10.3 b
5.0	60 b	173 c	6.4 b	10.3 b
7.5	110 ab	284 b	6.6 b	10.7 b
10.0	94 ab	210 c	5.8 b	9.6 c
F. test	*	**	*	*

*and ** indicate significant differences at P < 0.05 and P < 0.01 respectively, according to F. test. Means followed by a common letter in the same column are not significantly different at the 5 % level.

The irradiation could have decreased the auxin content. Auxin determines the mitotic activity in the callus and is considered the main plant hormone required for the activation of cell division *in vivo* and *in vitro*. This decrease in auxin content could result in slow growth of callus (Abdel-Maksoud, 1992). High doses of gamma irradiation reduce number and/or length of cells (Badr *et al.,* 1978). However, Al-Safadi and Simon (1990**)** reported that cell size and cell number of carrot callus increased after exposure to various gamma irradiation doses, i.e., 0.5- 40 kr. Generally, gamma irradiation affects callus diameter through regulation of auxin activity in culture medium and hence regulating plant growth, morphogenesis and cell elongation (Dietz *et al.,* 1990).

2.2 Effect of gamma rays on plantlets regeneration

Table 4 indicates that there were highly significant differences among gamma irradiation doses for number of embryos. Irradiation dose at 5 Gy produced the highest number of embryos, while the lowest number of embryos was obtained from 2.5 Gy. No embryos or plantlets were obtained from 10 Gy.

Irradiation dose at 7.5 Gy gave the highest number of normal plantlets and total number of plantlets per callus, while the control gave the lowest one. The highest percentage of normal plantlets was exhibited by the control. Zehn (1998) reported that the number of globular bodies (were capable of differentiating into plantlets) of garlic leaf callus decreased at the higher radiation doses (10 Gy). Generally, the high doses of irradiation may lead to a sharp decrease in auxin content in metabolically active tissues (Croci *et al.* 1990), which is essential to the formation of embryogenic cells. On carrot, it has been recorded that the higher doses of gamma irradiation inhibited shoot formation (Al-Safadi and Simon, 1990). This may be due to increased rate of lethality, injury and cloves sterility in garlic with increasing doses of gamma irradiation (Selvara *et al.* 2001).

The control produced the lowest number of abnormal plantlets (0.3), while the 7.5 Gy treatment produced the highest number of abnormal plantlets per callus (3.4). However, the 5 Gy treatment produced the highest percentage (61.25%). Our results indicate that the number of abnormal plantlets increased with increasing gamma irradiation doses. Danyansagar and Choudhary (1978) found that the frequency of mutations increased as the dose of irradiation was increased up to 7 Gy and higher doses had a lethal effect on garlic plants. In this respect, Croci *et al.* (1994) concluded that DNA was the sensitive cellular constituent to radiation in garlic. Several mutations in garlic plants which appeared after treating garlic cloves with gamma rays such as tall mutant, dwarf mutant and chlorophyll deficiency were the most frequently observed mutations.

Table (4): Effect of gamma rays on plantlets regeneration of irradiated cloves.

Gamma rays (Gy)	Number of embryos/ callus	Total number of plantlets/callus	Normal plantlets		Abnormal plantlets	
			No./callus	%	No./callus	%
0	13.73b	1.60 d	1.30 bc	79.13 a	0.3 c	20.87 c
2.5	4.87 d	3.13 bc	2.33 b	74.47 b	0.8 bc	25.52 c
5.0	18.86 a	3.27 b	1.27 bc	38.87 d	2.0 b	61.25 a
7.5	7.60 c	8.00 a	4.60 a	57.50 d	3.4 a	42.5 b
10.0	0.00 e	0.00 e	0.00 d	0.0 e	0.0 d	0.0 d
F. test	**	**	**	*	**	*

*and **. indicate significant differences at P < 0.05and P < 0.01, respectively, according to F. test. Means followed by a common letter in the same column are not significantly different at the 5 % level.

Generally, callus proved to be more resistant than cloves or other plant organs to gamma irradiation. This could be explained upon the fact that plant organs contain active meristematic cells, which provide a repair system (Maluszynski *et al.,* 1995).

3. Cytological analysis:

Table 5 shows the mitotic index and different cases of abnormalities of the callus regenerated plants and cloves after treatment with different gamma rays doses. A range of variation for each of mitotic index and cases of abnormalities of all regenerated plants was observed compared with the control plants.

Concerning the mitotic index, the values of treated cells were lower than the control ones. The percentage of chromosomal abnormalities increased with increasing the irradiation dose. The irradiated plants exhibited one or more types of mitotic chromosomal abnormalities **s**uch as non-oriented chromosome**,** bridges, lagging polyploidy, fragmentation, and nucleoli at different mitotic stages (Fig 3). The results indicated that a few number of the bridges and lagging were found in the investigated cells, while a high number of cells with fragmentation, stickiness and nucleoli were specifically observed at higher doses of radiation.

These results are in agreement with the findings of Al-Safadi and Simon (1990) who reported a positive correlation coefficient between dose and mitotic abnormalities in carrot callus. They also added that tissue culture conditions increased the abnormalities in callus compared to root tips. Also, the study of Talavera *et al.* (2003) indicated that gamma irradiation induced different kinds of chromosomal aberrations such as lagging, bridges and multinuclei in garlic. The abnormalities may be produced by ionizing irradiation that interferes with the normal process of cell division. This interference with cell division is the most immediate effect of ionizing irradiation on the transmission of the genetic material. Ionizing irradiation, in addition, causes lagging of chromosomes on the spindle at anaphase, an effect ascribed to changes brought about in the centromeres. Ionizing irradiation promotes cross-over in the region of the centromere due to an effect of radiation on the centromere (Florencio *et al.,* 2004).

4. Esterase and peroxidase isozyme banding patterns

In this study, esterase and peroxidase isozyme patterns were used to demonstrate the existing variability among garlic clones because they are immediate products of structural genes and easily detectable. Thus, their variations (isoforms) are often associated with genetic differences.

Fig 4 indicates the differences between clones from callus and cloves irradiation in esterase banding patterns through tissue culture, as compared to the control. The control clones exhibited two bands (0.219 and 0.62 RF) while, clones produced from irradiated callus at 2.5 Gy exhibited three bands (0.319, 0.61 and 0.62 RF). On the other hand, clones produced from irradiated callus at 7.5 and 10 Gy exhibited three bands (0.333, 0.61 and 0.62 RF), whereas the clones produced from irradiated callus at 5 Gy exhibited two bands (0.247 and 0.62 RF). In clones produced from irradiated cloves, treatments with doses of 5 and 7.5 Gy exhibited the same two bands (0.319 and 0.62), while those clones produced from 2.5 Gy exhibited two bands (0.319 and 0.64).

Fig 5 shows that there were differences in peroxidase banding patterns and color intensity between clones produced from either irradiated callus or irradiated cloves as compared with the control. In clones produced from callus irradiation, the control and clones produced after the treatment with doses of 2.5, 7.5 and 10 Gy showed two bands, while clones produced after treatment with 5 Gy showed one band only (0.066 RF). In clones produced from irradiated cloves, clones produced from the doses of 2.5, 5 Gy and 7.5 Gy showed one band only (0.059, 0.039 and 0.059 RF), respectively.

Table (5): Mitotic index and chromosomal abnormalities of the regenerated plants obtained from irradiated and non irradiated callus and cloves.

gamma rays (Gy)	Irradiated callus		Irradiated cloves	
	Mitotic index	Abnormalities (%)	Mitotic index	Abnormalities (%)
0	9.7	4.3	9.7	4.3
2.5	8.2	6.3	2.6	5.2
5.0	7.7	9.1	4.2	7.8
7.5	8.1	12.8	6.6	10.8
10.0	7.4	14.5	-	-

- No plantlets were obtained

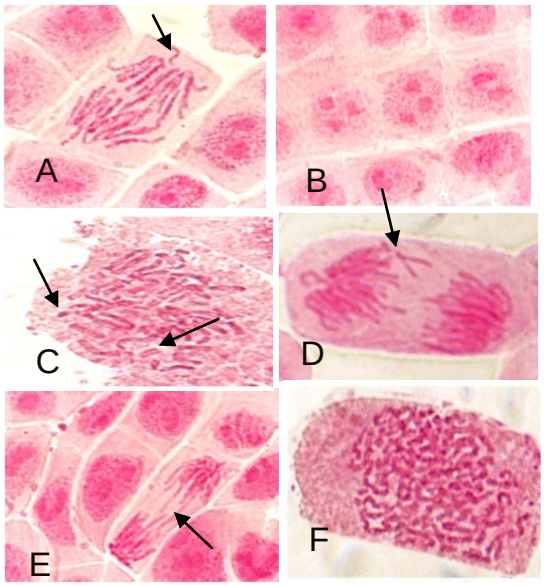

Fig.(3): Types of abnormalities scored in plants regenerated from irradiated callus and cloves of Balady cv. (A: Non oriented chromosome B: Nucleoli C: Fragments D:Lagging chromosome E:Chromosome bridge F: Polyploidy)

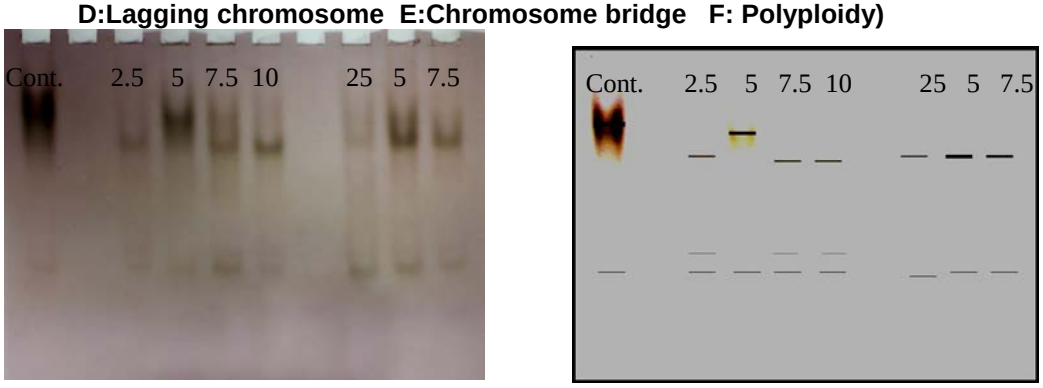

Fig. (4): Esterase isozyme banding patterns in irradiated and non-irradiated clones obtained from tissue culture technique.

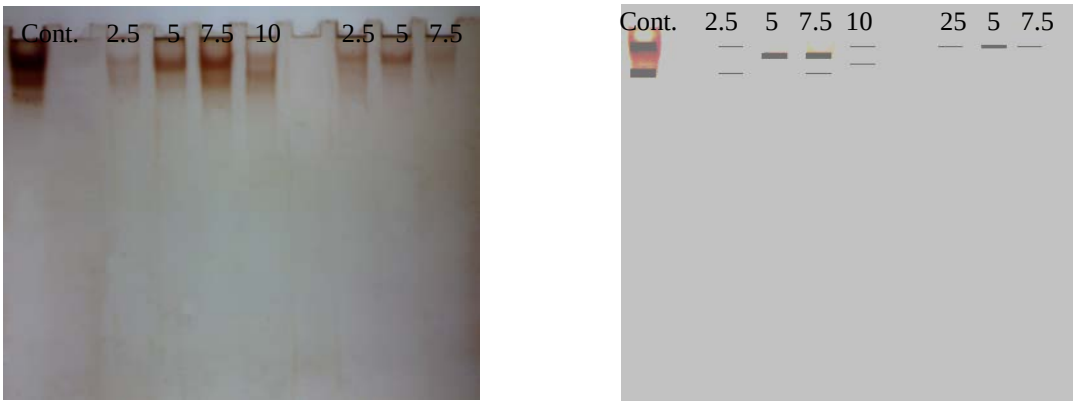

Fig. (5): Peroxidase isozyme banding patterns in irradiated and non-irradiated clones obtained from tissue culture technique.

Lage *et al.* (2001) emphasized that gamma irradiation induces changes in peroxidase isozyme profiles when compared with the sweet potato control. Generally, the detrimental effect of irradiation resulting in related growth is mainly due to the production of free radicals. The oxidation of biomolecules by free radicals which damage their structure are generated, following DNA repair, resulting in a variety of aberrations. Thus, they can cause DNA and protein damage due to which the membranes become leaky and mineral exchanges are hampered. The nuclear membrane is also destroyed and these reactive substances then enter the nucleus and cause oxidation of purines and pyrimidines, thus affecting the very basic genetic code (Walden and Schnell, 1990).

Conclusion

Our findings indicate that *in vitro* irradiation plays an important role in the breeding program of Egyptian garlic. The best irradiation dose to induce plantlets was 5 and 7.5 Gy for calluses and cloves, respectively. However, irradiation of calluses and cloves using 5 Gy resulted in a high percentage of abnormal plantlets. Electrophoresis analysis for both esterase and peroxidase isozymes indicated genetic variations in regenerated plants from Balady cultivar using gamma irradiation.

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إنتاج طفرات من الثوم عن طريق المعاملة بأشعة جاما وزراعة الكالس

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الملخص العربى

أجريت هذه الدراسة بهدف إنتاج تباينت وراثية فى الثوم (الصنف البلدى) عن طريق تعريض الفصوص أو الكالس إلى جرعات مختلفة من أشعة جاما (صفر ، ٢٥ ،٥ ، ٧٥ ، ١٠ جراى). اوضحت النتائج أن تعريض

الكالس الى ٥ جراى أدى إلى إنتاج أكبر عدد من النباتات لكل كالس ، بينما أعطت معاملة الكنترول أقل عدد من النباتات لكل كالس. كما أعطت معاملة الفصوص ب ٧٥ جراى الى الحصول على أعلى عدد من النباتات لكل كالس.

تم الحصول على أعلى نسبة مئوية للنباتات غير الطبيعية والتباينات السيتوبلازمية عندما تم تعريض الكالس والفصوص الى ٥ جراى. وقد تم ايضا دراسة تحليل الهجرة الكهربائية لمشابهات الانزيمات لكل من إنزيمى االستريز والبيروكسيديز للنبتات الناتجة من معاملة الكالس والفصوص بأشعة جاما