FACULTY OF AGRICULTURE

Minia J. of Agric. Res. & Develop.
Vol. (36), No. 2, pp. 271-292, 2016

INDUCED VARIATION IN SOME HORTICULTURAL AND CYTOLOGICAL CHARACTERISTICS OF TWO GARLIC (*Allium sativum* L.) Genotypes

Asmaa S. Ezzat[1]; Yasser M.M. Moustafa[1]; Kasem Z. Ahmed[2]; Mohamed M. Farrag[1] and Seifel-Nasr H. Gadel-Hak[1]

[1]Horticulture Department, Faculty of Agric., Minia Univ., Minia, Egypt.
[2]Genetics Department, Faculty of Agric., Minia Univ., Minia, Egypt.
Corresponding author: E-mail: yasser.mostafa@mu.edu.eg

Received: 4 May (2016) Accepted: 17 July (2016)

ABSTRACT

Garlic (*Allium sativum* L.) is a consumed vegetable crop worldwide. Clonal selection of garlic clones is the main breeding method because plant sterility precludes crop improvement through cross hybridization**.** The genetic variation can be increased in garlic by alternative approaches such as somaclonal variation, mutagenesis and genetic transformation. Therefore, in this study, different doses of gamma irradiation (2, 8, 12, 16, 20, and 24 Gy) as well as control plants were applied to induce variabilities. Their effect on some cytological, morphological and horticultural characteristics of two garlic genotypes (White Clone and cultivar Egaseed 1) was assayed at the first mutated generation (M_1V_1) in two seasons. The results showed that the highest dose of gamma radiation increased the total mitotic chromosomal abnormalities. The cytological recorded aberrations included chromosomal and chromatid bridges, chromosomal breaks and fragments, lagging and micronuclei. The higher doses of gamma rays (16.0, 20.0, and 24.0 Gy) increased the rate of lethality and decreased garlic plant growth compared to the non-treated control plants. The low doses resulted in higher values of growth characteristics (e.g., percentage of germination, plant height and number of leaves/plant). The average germination percentage under laboratory and emergence under field conditions, plant height, number of leaves/plant and fresh weight were higher when the cloves of both studied genotypes were exposed to 2 Gy dose. The lowest

bulbing ratio was noticed for both genotypes treated with the 2 Gy treatment. By evaluating the horticultural behavior and cytological profiles of the treated garlic genotypes, some of the tested treatments could be used to develop desirable genetic variability in garlic. Two Gy could be used as a stimulated pretreatment in garlic production.

Keywords: Garlic, Chromosomal aberrations, Gamma irradiation, Mutation, First mutated generation (M_1V_1), Clonal selection.

INTRODUCTION

Garlic (*Allium sativum* L) is a member of *Liliaceae* which included onions, shallots, and leeks. It is a worldwide important vegetable crop (Nagovreny, 1998 and Panthee *et al*., 2006). Furthermore, large quantities of garlic are used for pharmaceutical purposes (Kik and Gebhardt, 2001), whereas, garlic extract has been used as a traditional medicine for the prevention and treatment of cardiovascular disease (Ackermann *et al.*, 2001).

Garlic is a diploid obligate apomicts ideal crop that is primarily propagated asexually from cloves (Bradley *et al*., 1996). Clonal selection is the main breeding method for garlic (Lampasona *et al*., 2003 and Abdel-Rasheed *et al*., 2016). For centuries, garlic has been propagated clonally, which may have resulted in a low variability. But, clonal lineages within this species show a remarkably high degree of phenotypic diversity (Ata, 2005 and Osman *et al*., 2007). Therefore, to improve the existant cultivars, modern and biotechnological techniques such as somaclonal variation, mutagenesis or genetic transformation could be used.

Common garlic cultivars as reported by Bozzini (1991) have a somatic chromosome number of 2n=16 chromosomes and some garlic clones were shown to be tetrapliod (4n=32) although some cultivars might be triploid. However, chromosomal aberrations are common in garlic due to multiple translocations which are sometimes involving 8 or even 10 chromosomes and some sterile varieties have a normal karyotype (Sanai and Davis, 1967 and Osman *et al*., 2007). Mitotic index is one of the mitotic parameters which reflect the genetics control system of division and the existence of chromosomal aberrations in several organisms including garlic (Swanson *et al*., 1990 and Kaushik, 1996).

Gamma radiation induced genetic variability in plants. The first experiments on caryopses exposed to relatively low doses of ionizing radiation in order to stimulate growth and development were performed a few years after the discovery of X-rays as reported by Focea *et al*. 2012. Such exposure stimulated seed germination, plantlet growth, flowering, plant size and yield as reviewed by Breslavets (1946). These results allowed Pateskevich (1961) to claim that the irradiated seeds prior to sowing may represent a great promise from the viewpoint of its practical application in agriculture. Several researchers concluded that low doses

of gamma rays stimulate cell division, growth and development in various organisms. This phenomenon has been analyzed and discussed by several authors in different plant species (Luckey, 1980; Shalaby *et al*., 1983 a and b; Sagan, 1987; Planel *et al*., 1987; Korystov and Narimanov, 1997). Beside gamma rays, ethylmethan sulfonate (EMS) as mutagenesis may be considered a reliable alternative breeding tool (Batchvarov, 1993). Shalaby *et al*. (1983 a and b) found that in the M_1V_2 generation, gamma radiation and EMS treatments increased the variability of garlic plants with respect to some characters such as bulb weight, clove weight and harvest time. Alverez *et al*. (1996) found that different doses of gamma–ray induced different phenotypic variations in the second mutated garlic generation (M_1V_2). Also, Kumar and Tiwari (1998) mentioned that various doses of gamma-rays in some garlic genotypes increased the time period from sprouting to harvest but reduced plant height, leaf length, bulb and nick diameters in the M_1V_1 generation. Iglesias- Enriquez *et al*. (2001) found that higher doses of gamma-irradiation in garlic and onion inhibited cloves and seeds germination. Moreover, garlic bulb and clove characteristics were significantly influenced by the application of two doses of gamma rays (2.5 and 5.0 Gy) as reported by Selvaraj *et al*. (2001) and Hemada *et al*. (2012).

The present study was conducted to evaluate some horticultural and mitotic chromosomal characteristics in the M_1V_1 plants of two garlic genotypes (White Clone and Egaseed1) exposed to gamma rays in two successive seasons.

MATERIALS AND METHODS

Plant materials and gamma radiation treatments

Bulbs of two garlic genotypes (White clone and Egaseed1 cultivar) were kindly obtained from Sids Research Station, Agricultural Research Center, Beni Suef Governorate, Egypt. Both genotypes were subjected to gamma irradiation treatments. The semi-dry cloves of both genotypes were exposed to six different doses of gamma radiations (2, 8, 12, 16, 20, and 24 gray "Gy") using a Canadian Cell-40 Cs^{137} with dose rate one gray (Gy)/2.14 min or 0.758 Rad/sec. Radiation was carried out at the National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt. The control cloves were treated with distilled water. The experiments were done in two successive seasons of 2013/2014 and 2014/2015.

Laboratory experiment

Some cloves of the first mutated generation (M_1V_1) as well as the control were germinated *in vitro* (on MS hormone free medium, Gadel-Hak *et al.*, 2011) or in distilled water to use their roots in the cytological studies. Also, three replicates of five cloves per each treatment were germinated on moistened filter paper and then germination percentage and number of roots per clove under room

temperature conditions were calculated. Germination and rooting were determinated after 5-7 days from planting by success of clove tip and root appearance of > 0.5 cm. Data recoded under the laboratory conditions for the following characteristics.

1 Laboratory Data.

1.1.Germination percentage (GP%): Germination percentage (%) was calculated for the treatments and control cloves as**:**

GP (%) = (Number of germinated cloves / total number of tested cloves) X100

1.2.Number of roots / clove: Roots of > 0.5 cm per clove was counted and their average was used.

1.3.Cytological Studies and mitotic analysis

The cytological studies were carried out in the Cytogenetic Lab., Department of Genetics, Faculty of Agriculture, Minia University, El-Minia, Egypt. For mitotic studies and recording some mitotic parameters of both genotypes, growing root tips of 1-2 cm long were collected and pretreated in 0.05% colchicine solution at room temperature for three hours and immediately fixed with the Farmer's Fixative Solution (absolute ethyl alcohol and glacial acetic acid 3:1, v/v) for 24 h and stored in 70% ethanol at 4^{o}C, rinsed with water and hydrolysis for 5 min in 1N HCl at 60^{o}C was done and staining was then performed with 0.5% acetocarmine for root tips of 3 cloves per each treatment of both genotypes. The different mitotic stages were observed under a light microscope. Good dividing cells were photographed using (CCD Camera Olympus C-4040). Cells with clear mitotic chromosome irregularities were scored at prophase, metaphase, anaphase and telophase stages of all division and some good images were taken and recorded.

2. Field Experiment.

Irradiated M_1V_1 cloves at two days later and their controls of both genotypes (White clone and Egaseed1 cultivar) were cultivated on 30th of September of the two successive seasons of 2013/2014 and 2014/2015 at the Experimental Farm of Horticulture Dept., Fac. Agric., Minia University, Minia, Egypt. in a Randomized Complete Block Design (RCBD) with three replications. Each treatment was planted in a single row (3.5 m long X 60 m wide) and cloves were spaced 10 cm apart in each row. Agricultural practices were applied at the proper stages of development as recommended by the Agriculture Research Center (ARC), Egypt, No. 521/1999. Measurements were made on single plant basis for the following traits:

Field Data.

2.1 Emergence %: The emerged cloves above soil surface were recorded daily and the percentage was calculated by dividing emerged cloves by the total planted cloves.

2.2 Plant height (cm): Plant height was measured for all growing plants (in cm) from the bulb apex to the top of the longest leaf plade after 30, 60, 90, and 120 days from planting.

2.3 Number of leaves/plant: Number of leaves for each individual garlic plant was counted after 30, 60 and 90 days from planting.

2.4 Bulbing ratio: Neck and bulb diameters of all tested garlic plants were recorded using a calipers after 120 days from planting and at harvesting time. Then, the blubing ratios were calculated by dividing the neck diameter by bulb diameter (neck diameter/bulb diameter) as reported by Mann (1957).

2.5 Leaf length and width (cm)**:** These two characteristics were measured for the fourth leaf from the soil surface using a ruler for each individual plant. The leaf width was determined at the broadest site of leaf blade.

2.6 Plant fresh weight (g): Whole fresh garlic plants from all treatments were weighed (in grams) after harvesting and their average were used for statistical analysis.

Statistical analysis.

All recorded data either in lab or in field were subjected to the analysis of variance (ANOVA) and all means were compared using L.S.D test or Duncan's Multiple Range Test (DMRT) **(Duncan, 1955)** at 0.05 level of probability as described by **Gomez and Gomez (1984)**. The MSTAT-C program version 4 (Michigan University, USA) was used.

RESULTS AND DISCUSSION

The present work includes the studying of different parameters i.e. percentage of clove germination and number of roots/clove along with the cytological studies at laboratory. While other morphological and agronomical characters including emergence percentage, plant height, number of leaves, leaf width, leaf length, bulbing ratio, plant fresh weight were recorded on plants grown under the open field conditions.

1. Laboratory experiments

1.1 Germination percentage (%)

The percentages of clove germination of the two tested garlic genotypes (White Clone and cultivar Egaseed1) treated with five doses of gamma radiation are showed in Table 1 and Fig. 1. In general, four out of the five tested doses resulted in germination percentage values of 100% in M_1V_1 for both genotypes. Also, the results showed that 24 Gy of gamma radiation treatment gave zero germination percentage for the two studied garlic genotypes while healthy germinated plantlets were observed with 2 Gy treatment as shown in Fig. 1 for both genotypes. Generally, gamma treatment with doses higher than 20.0 Gy inhibited the germination process in both genotypes. These results are in agreement with those reported by Badr *et al.* (1978) and Ieglesias - Enriquez *et al*. (2001) and Hemeda *et al*. (2012) who found that higher gamma rays doses inhibit germination in garlic. Data showed that plants derived from irradiated cloves exposed to higher doses > 16 Gy did not survive, so it was not possible to follow up these treatments.

Table (1). Average effect of gamma radiation doses (0.0, 2.0, 8.0, 12.0, 16.0, 20.0 and 24.0 Gy) on germination percentage and number of roots/clove in the first mutated generation (M_1V_1) of two garlic genotypes (White Clone and cultivar Egaseed1) under laboratory conditions

Gamma rays dose (Gy)	White Clone		Egaseed1	
	Germination Percentage	No. of Roots/Clove	Germination Percentage	No. of Roots/Clove
Control	100 A	6.33 B	100 A	6.33 BC
2.0 Gy	100 A	7.67 A	100 A	10.0 A
8.0 Gy	100 A	6.33 B	100 A	6.67 B
12.0 Gy	100 A	4.33 C	100 A	5.00 CD
16.0 Gy	100 A	3.00 D	100 A	3.667 D
20.0 Gy	100 A	1.667 E	100 A	2.00 E
24.0 Gy	Zero B	Zero F	Zero B	Zero F

In each column, means followed by the same letter are not significant at the 0.05 level by Duncan's Multiple Range Test (DMRT).

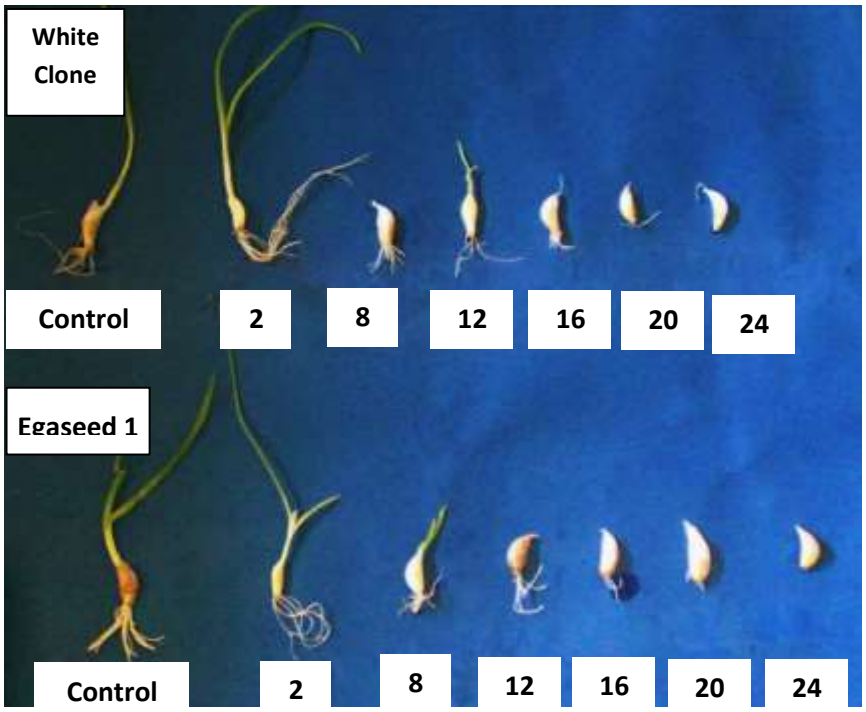

Fig 1: Effects of gamma radiation doses (0.0, 2, 8, 12, 16, 20, and 24 Gray) on the germination percentages of two garlic genotypes (White Clone and cultivar Egaseed 1)

1.2 Number of roots/clove

The effect of gamma radiation on the number of roots/clove of the two garlic genotypes during the first mutated generation (M_1V_1) is presented in Table (1). There were significant differences among the tested doses. It is generally noticed that the number of roots decreased by increasing gamma radiation dose above 2 Gy dose compared with the control. The stimulation effect of the low dose of gamma rays was reported by several authors in different plant species (Planel *et al*., 1987; Sagan, 1987; Selvaraj *et al*., 2001 and Focea *et al*., 2012). Low doses of radiation stimulate physiological process in plants (Focea *et al*., 2012).

1.3 Cytological Studies

Mitotic studies in the present work were confined to the examination of certain morphological features of prophase, metaphase, anaphase, mitotic index and total abnormalities among cell development stages of garlic cells derived from cloves exposed to 5 different doses of gamma rays using two garlic genotypes (White Clone and cultivar Egaseed1).

1.3.1 Mitotic Index (MI)

The statistical analysis (ANOVA) and the LSD values at 0.05 level were made for mitotic index, prophase index, metaphase index , anaphase and telophase index (Table 2). Data as shown in Table (2) showed that MI values were significantly higher with 16.0 Gy in both genotypes than those observed in the control. The highest value in the examined cells of the cultivar Egaseed1 cloves was 27.06% and it was 19.98% in the white clone. Cloves treated with 8.0 Gy dose of gamma irradiation induced the lowest MI values in White Clone (14.22%). Results of the mean percentages of the different mitotic stages prophase, metaphase and anaphase and telophase are shown in Table (2). In both genotypes values were recorded using number of examined cells ranged from 4155 to 10148 in plants of White Clone (Table 2) and from 3728 to 5318 cells in cultivar Egaseed1 (Table 3).

Percentages of cells at prophase

Data revealed that the highest percentages of prophase index were found in plants treated with 12.0 Gy (52.17%) for White Clone. Insignificant differences were detected among the tested treatments in cultivar Egaseed1 (Table 2).

Percentages of cells at metaphase

Significant differences among some of the tested treatments in cultivar Egaseed1 were shown in Table (2). The highest value of metaphase index in the cultivar Egaseed1 (32.68%) and in White Clone (31.89%) were obtained from roots derived from 12.0 Gy treatment, while the lowest value (23.31%) was obtained with control plants for Egaseed1. In the White Clone, the lowest value of metaphase index was given from roots treated with 8.0 Gy (22.97%) with insignificant differences among the tested treatments.

Percentages of cells at ana & telophase

The highest values of ana & telophase were 23.81% for Egaseed1

and 43.33% for White Clone (Table 2). These values were obtained in the examined roots of cloves treated with 2 Gy in Egaseed1 and the White clone. The lowest value was scored with cloves treated with 8 Gy in cultivar Egaseed1 (13.07%) but it was and (15.93%) in the white clone with 12 Gy. The effect on the ana-telophase stage can be utilized as test protocols for testing the radiation sensitivity of garlic as reported by Datta (2002) and Datta *et al*. (2012).

1.3.2 Abnormalities (Mitotic irregularities)

The recorded mitotic aberrations are detected at prophase, metaphase, anaphase and telophase, mitotic stages as shown in Table 3 and Fig. 2. The major chromosomal abnormalities observed were bridges, fragments, and micronuclei. Also, clumping, laggards, exclusion were observed and photographed as shown in Fig 2. Data in Table 3 showed that the highest values of the chromosomal abnormalities (bridges and fragments) were detected in the roots of cloves treated with 16 Gy (9.46%) for White Clone and (12.57%) for cultivar Egaseed1. While, the lowest values were observed in roots treated with 2 Gy for both genotypes. The highest dose of gamma radiation increased the total mitotic chromosomal abnormalities. These results are in good agreement with previous studies by Patil and Bhat 2000 and Kumer *et al*. (2015).

Table (3) showed that only cells from cloves of the White Clone treated with 2 Gy exhibit micronuclei compared to all other treatments in both genotypes. Croci *et al*. (1991) revealed that the treatment with gamma irradiation (10 Gy) of post dormant garlic cloves induced significant inhibition in sprout growth. Likewise, 20 Gy dose of gamma rays induced morphological and cytological changes (micronuclei, chromosomal aberrations and reduction of mitotic index) in onion (Vaijapurkar *et al*., 2001 and Datta *et al*., 2011). However, the chromosomal aberrations like micronuclei, multibridges, and fragments were not incorporated to the main nucleus during cell division cycle as reported by Fennech (2002). The chromosomal fragments are more common in metaphase and anaphase (Kumar *et al*., 2015). In the present study, fragmented chromosomes were observed in metaphase mitotic stage (Table 3). These results agreed with the previous results of Grant (1978); Patil and Bhat (2000) and Kumar *et al*. (2015).

2. Field experiment

The two garlic genotypes were planted in both seasons on 30th of September, to study and evaluate the effect of five different doses of gamma rays as well as the untreated control on some growth characters, clove emergence (%), plant height (cm), number of leaves/plant, leaf width (cm), leaf length (cm), bulbing ratio and fresh weight (g). The recorded data were as follow:

2.1 Emergence %

The percentage of cloves emergence of the two tested garlic

genotypes (White Clone and cultivar Egaseed1) treated with five doses of gamma radiation is shown in Fig 3 and Fig 4. In general, the results showed that 24 Gy of gamma radiation treatment gave zero% value, for the two studied garlic genotypes, while, high emergence percentage was given by 2 Gy treatment for both genotypes (93.3% for White Clone and 100% for Egaseed1). Generally, gamma treatment with doses higher than 16 Gy inhibited the emergence process in both genotypes. Badr *et al.*, (1978), Ieglesias—Enriquez, *et al*. (2001) and Hemada *et al*. (2012) concluded that higher gamma rays doses inhibited emergence in garlic. Cloves exposed to higher doses (> 20 Gy) did not survive, so they are not possible to follow up these treatments as shown in Fig. 3 and 4.

2.2 Plant Height

Data in Table (4) showed the significant effect of radiation doses on plant height character of the studied garlic genotypes. The plant height of White Clone was 26.4 cm with 2.0 Gy after 30 days from clove plantation. While, after 60 days it was 62.8 cm and reached after 90 days to 80.5 cm and it was 94.4 cm at harvesting. For cultivar Egaseed1, results in Table 4 showed that plant height with 2.0 Gy treatment was 27.4 cm after 30 days, 45 cm after 60 days, 68.5 cm after 90 days and 94.8 cm at harvesting. The lowest values of plant height at the harvesting time were detected with 16.0 Gy treatment in both genotypes. In the second season the lowest values were detected with 12.0 Gy in both genotypes. Increasing the gamma radiation dose decreased the plant height significantly (Table 4). Theses results in agreement with previous studies conducted by Esnault *et al*. (2010); Hassan *et al*. (2015) and Hemada *et al*. (2015)

2.3 Leaf characteristics

The mean numbers of leaves/plant after 30 and 90 days from cultivation are shown in Table 5 and Fig 5, for the White Clone and cultivar Egaseed1. The highest number of leaves was observed in plants derived from irradiated cloves with 2 Gy. There were 4.7 leaves/plant for White Clone and 3.6 leaves/plant for the Egaseed1 cultivar. While, the number of leaves/plant from cloves exposed to 8, 12 and 16 Gy significantly decreased. After 90 days from cultivation the highest value was 12.0 and 12.1 leaves for White Clone and 10.8 and 10.1 leaves for Egaseed1 with 2 Gy in the first and second seasons, respectively. Exposure to dose > 2Gy decreased significantly the average leaf width of both genotypes. The maximum leaf lengths were 48.8 cm and 43.0 cm which recorded in plants of White Clone and Egaseed1 when their seed cloves were exposed to 2 Gy. On the other hand, insignificant difference was found between leaf length at plants exposed to 2 Gy and control treatment in the White Clone. Growth inhibition by higher doses of radiation treatments was attributed to cell cycle arrest in the M_1V_1 growth stages during somatic cell division (Esnault *et al*., 2010) and /or to variety of damages in the entire genome

(Preuss and Britta, 1979). The highest doses of gamma rays decreased plant height and leaves number compared to control. This was in agreement with previous studies by Shalaby *et al*. (1983 a), Hammad *et al*. (1988) and Hemada *et al*. (2012).

2-4 Bulbing Ratio and plant Fresh weight (g)

Bulbing ratio ≤ 0.5 is reflecting the time of bulb initiation as mentioned by **Mann (1957)**. Table 6 and Fig 5 showed the effect of gamma rays doses on bulbing ratio and plant fresh weight traits. The highest and lowest percentages of bulbing ratio after 90 days from cultivation was 0.61 with zero Gy treatment followed, in descending order, by 12, 8 and 16 Gy treatments. The 2 Gy dose scored 0.234 for the White Clone. For cultivar Egaseed1, the highest value was 0.64 with 16 Gy treatment followed by zero, 12, 8 and 2 Gy treatments which scored 0.302. At harvesting time, the highest value was 0.31 with 16 Gy treatment followed by 12, zero, 8 and 2 Gy treatments. The lowest value was 0.14 for the White Clone. In Egaseed1, the highest value for bulbing ratio was (0.46) with 16 Gy treatment while gamma dose 2 Gy scored the lowest value (0.18). In the second season the untreated cloves produced true bulbs in both genotypes at the harvesting time On the other hand, bulbing ratio values in the control treatments were higher than 0.5 at 90 days age.

Regarding the whole plant fresh weight, value of the White Clone was 186.1 g with 2 Gy treatment followed in decreasing order by the control, 8, 12, 16 Gy treatments. On the other side, the highest value in Egaseed1 was 194.0 g with 2 Gy treatment followed by control treatment. These results agreed with previous results which indicated that low doses of gamma rays stimulate cell division, growth and development in various organisms not only in plant but also in animal. This might be due to hormonal signaling network in plant cell as suggested by several authors, related two different plant species (Luckey, 1980; Planel *et al*., 1987; Koristov and Narimanov, 1997 and Hassan *et al*. 2015).

The obtained results indicated that the highest doses of gamma rays decreased plant height and leaves number compared to control and these results were in agreement with previous studies (Shalaby *et al*., 1983 a and Hammad *et al*., 1988). Also, the high gamma radiation doses inhibited plant growth during the peak division and differentiation stage in garlic plant which agreed with previous results (Hassan *et al*., 2015).

CONCLUSION

In the present study, treatments of garlic cloves with different doses of gamma radiation induced plant variabilities at both cellular and whole plant levels which could be a suitable strategy in garlic breeding.

Table (2) Average values of the effect of gamma rays on mitotic index, and the percentages of mitotic stages in Egaseed1 and White Clone garlic genotypes at the first mutated generation (M_1V_1) after exposure to differences doses of gamma rays

Doses of gamma rays	Egaseed 1					White Clone				
	Total number of examined cells	Mitotic index %	Prophase %	Metaphase %	Ana& Telophase%	Total number of examined cells	Mitotic index %	Prophase %	Metaphase %	Ana& Telophase%
Control	4418	17.43	52.18	23.31	23.81	4155	17.64	50.00	25.36	24.62
2.0	4905	18.62	51.03	26.05	22.91	6902	17.39	28.21	28.44	43.33
8.0	4696	19.72	56.44	30.48	13.07	10148	14.22	34.08	22.97	42.92
12.0	3728	18.83	53.16	32.68	14.18	8102	17.83	52.17	31.89	15.93
16.0	5318	27.06	55.65	25.47	18.85	9216	19.98	45.21	31.48	23.41
LSD	_____	6.069	NS	6.657	7.351	_____	2.108	5.711	NS	12.00

Ns= not significant

Table (3) Average values of the effect of gamma rays on chromosomal abnormalities (bridges, fragments and micronuclei) in Egaseed1 and White Clone garlic genotypes at the first mutated generation after exposure to gamma rays (M_1V_1)

Doses of gamma rays	Egaseed 1					White Clone				
	Total Divided cells	Bridges	Fragments	Micronucli	Total Abnormalities	Total Divided cells	Bridges	Fragments	Micronucli	Total Abnormalities
Control	740	1.555	1.917	0.00	3.472	728	1.193	1.680	0.00	2.873
2.0	915	0.014	0.072	0.00	0.086	1214	0.00	0.00	3.673	3.674
8.0	963	0.987	1.850	0.00	2.837	1446	2.618	2.582	0.00	5.200
12.0	708	2.767	3.285	0.00	6.052	1443	2.985	3.293	0.00	6.277
16.0	1453	5.817	6.753	0.00	12.570	1866	5.065	4.394	0.00	9.462
LSD		0.6657	1.573	-	2.004		0.7192	0.6697	-	1.509

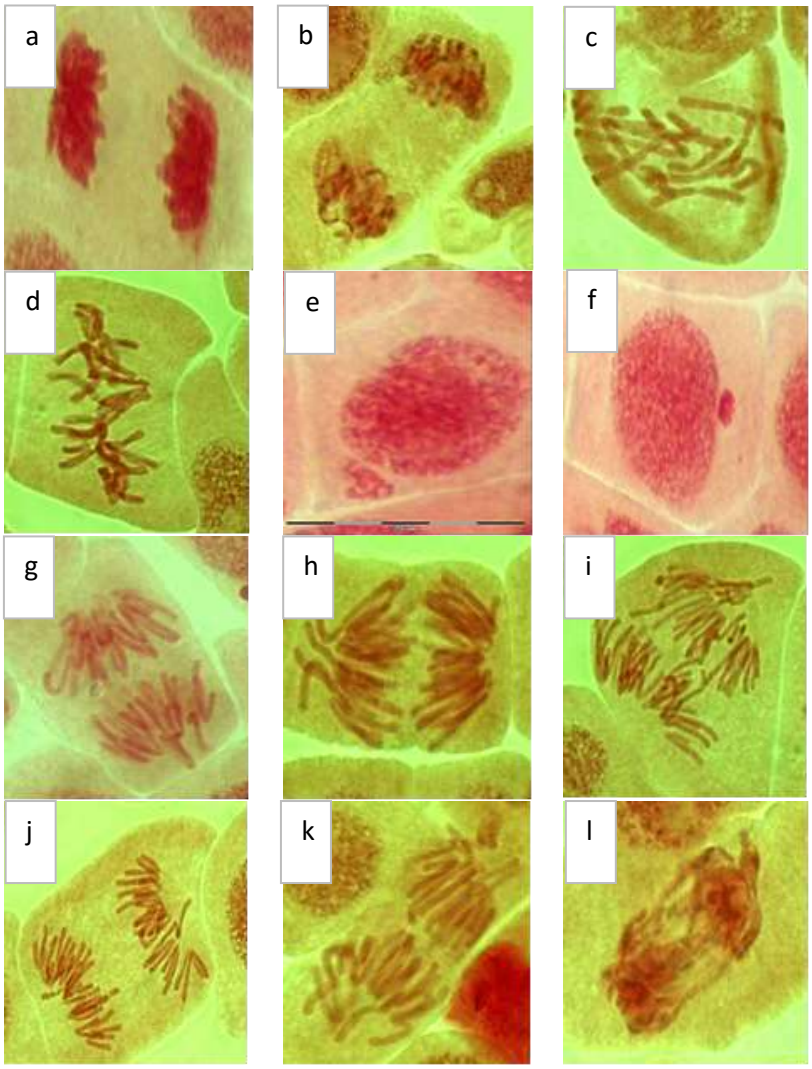

Fig (2): Mitotic chromosomes of two garlic genotypes showing (a),(b) normal telophase on different doses ;(c)-(d) normal metaphase, (e) microcyte, (f) micronuclus,-(g) -(h)-(i)-(j)-(k) normal and abnormal anaphase, (l) sticky chromosomes with multibridges

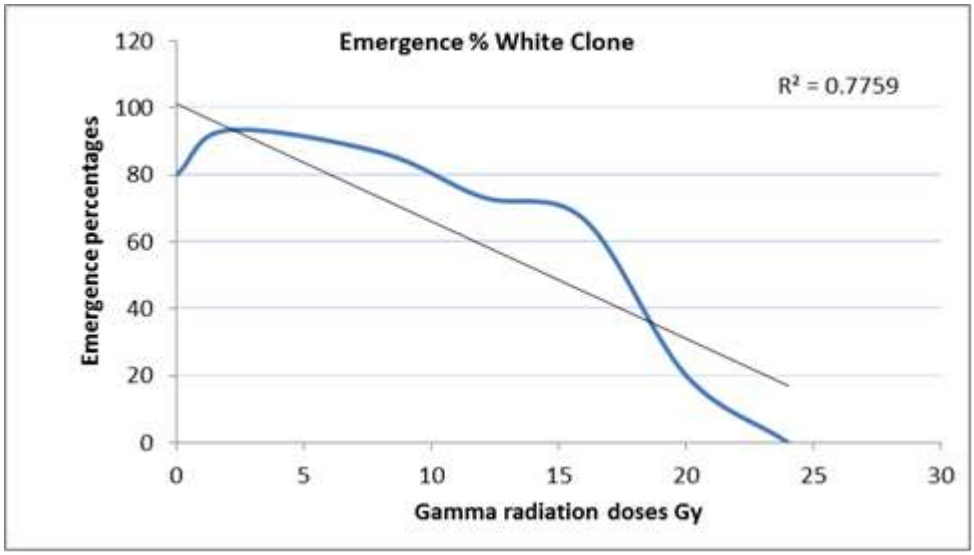

Fig 3: Average effects of gamma radiation on the emergence % of the garlic genotype (White Clone) under field conditions in the first mutated generation (M_1V_1) after exposure to gamma rays in the two successive seasons (2013/2014 and 2014/2015)

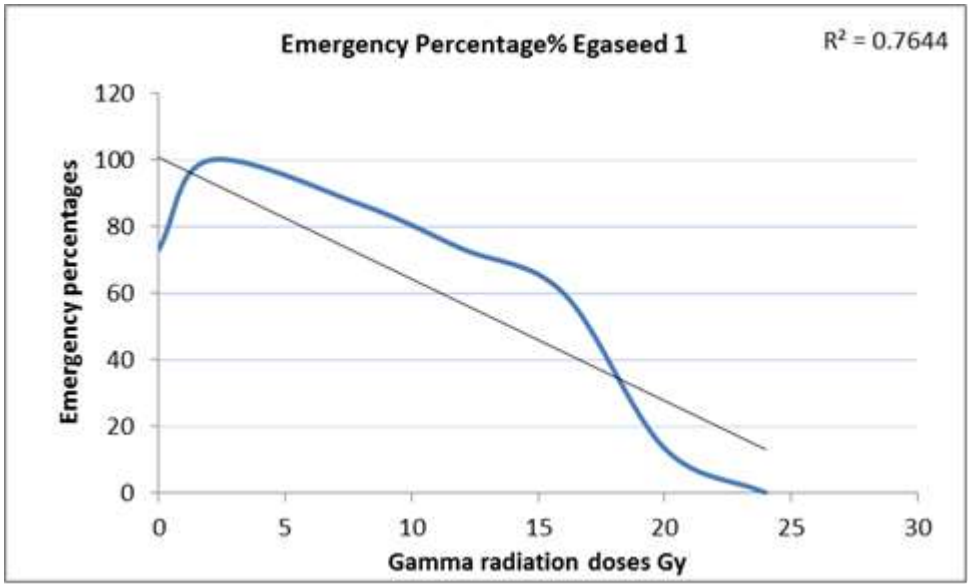

Fig 4: Average effects of gamma radiation on the emergence % of the garlic genotype (Egaseed1 Cultivar) under field conditions in the first mutated generation (M_1V_1) after exposure to gamma rays in the two successive seasons (2013/2014 and 2014/2015)

Table (4): Effect of gamma ray treatments (0.0, 2.0, 8.0, 12.0 and 16.0 Gray) on plant height (in cm) of two garlic genotypes (White Clone and cultivar Egaseed 1) in the winter seasons of 2013/2014 and 2014/2015

Doses of gamma Rays (Gy)	White Clone				Egaseed 1			
	After 30 days	After 60days	After 90days	At harvesting	After 30 days	After 60days	After 90days	At harvesting
First season (2013/2014)								
Control	23.8 B	39.2 B	63.8 C	84.6 D	24.6 B	40.4 B	54.4 B	73.0 B
2	26.4 A	62.8 A	80.5 A	94.4 A	27.4 A	45.0 A	68.5 A	94.8 A
8	22.6 B	38.8 B	55.1 C	76.2 C	18.4 C	34.2 C	50.4 C	75.0 B
12	23.6 B	34.2 C	38.0 D	68.2 D	14.8 E	26.6 D	40.1 D	69.0 C
16	19.6 C	40.6 B	40.7 E	61.2 E	16.6 D	27.0 D	37.6 E	65.4 C
Second season (2014/2015)								
Control	22.5 B	51.8 C	63.8 C	63.7 B	18.9 B	31.3 C	54.4 B	57.9 B
2.0	26.8 A	65.5 A	80.5 A	83.8 A	22.8 A	47.10 A	68.5 A	75.7 A
8.0	17.5 C	30.7 D	55.1 C	58.6 C	17.7 C	33.0 B	50.4 C	53.9 B
12.0	15.9 D	28.8 DE	38.0 D	41.5 E	11.4 D	19.8 E	30.4 D	33.9 E
16.0	15.8 D	27.7 E	40.7 E	44.6 F	12.8 E	24.7 D	25.1 E	40.9 D

In each column, means of each season followed by the same letter are not significant at 0.05 level of probability by Duncan' s Multiple Range Test (DMRT).

Table (5): Effect of gamma rays treatment (0.0, 2.0, 8.0, 12.0 and 16.0 gray) on some leaf characteristics of two garlic genotypes (White Clone and cultivar Egaseed 1) in the winter seasons of 2013/2014 and 2014/2015

Doses of gamma rays	White Clone				Egaseed 1			
	Number of leaves/ plant		Leaf width (cm)	Leaf length (cm)	Number of leaves/ plant		Leaf width (cm)	Leaf length (cm)
	After 30 days	After 90 days			After 30 days	After 90 days		
First season (2013/2014)								
Control	2.8 B	10.0 AB	3.25 A	40.4 B	3.1 A	9.0 B	4.06 B	39.2 B
2.0	4.7 A	12.0 A	3.51 A	48.8 A	3.6 A	10.8 A	4.12 A	43.0 A
8.0	28 B	9.2 BC	2.59 B	34.6 C	2.3 B	8.0 C	3.42 C	36.8 BC
12.0	2.2 BC	10.2 AB	2.35 B	32.0 D	1.8 BC	6.4 D	3.14 C	38.6 BC
16.0	2.0 C	7.0 C	1.77 C	24.7 E	1.3 C	5.4 E	2.98 D	36.0 C
Second season (2014/2015)								
Control	_____	9.3 C	_____	_____	_____	8.1 B	_____	_____
2.0	_____	12.1 A	_____	_____	_____	10.1 A	_____	_____
8.0	_____	10.4 B	_____	_____	_____	8.5 B	_____	_____
12.0	_____	10.0 B	_____	_____	_____	8.0 B	_____	_____
16.0	_____	6.2 D	_____	_____	_____	8.2 B	_____	_____

In each column, means of each season followed by the same letter are not significant at 0.05 level of probability by Duncan' s Multiple Range Test (DMRT)

____ = Not estimate

Table (6): Effect of gamma ray doses on bulbing ratio after 90 days from planting time and at harvesting time as well as plant fresh weight of two garlic genotypes (White Clone and cultivar Egaseed1) in the winter seasons of 2013/2014 and 2014/2015

Doses of gamma rays (Gy)	White Clone			Egaseed 1		
	Bulbing ratio after 90day	Bulbing ratio at harvesting	Plant Fresh weight, g	Bulbing ratio after 90day	Bulbing ratio At harvesting	Fresh weight (g)
First season (2013/2014)						
Control	0.61A	0.308 B	126.1 B	0.575 AB	0.337 C	186.7B
2.0	0.234 C	0.168 C	186.1 A	0.302 C	0.177 D	194.0A
8.0	0.511 B	0.302 B	125 B	0.540 B	0.337 C	141.7 C
12.0	0.542 AB	0.426 A	107.8 D	0.563 AB	0.470 B	103.9 D
16.0	0.245 B	0.454 A	77.8 C	0.641 A	0.505 A	44.5 E
Second season (2014/2015)						
Control	0.55 A	0.28 AB	140 B	0.61 AB	0.32 B	120 B
2.0	0.40 D	0.14 C	192 A	0.61 AB	0.18 D	181 A
8.0	0.36 E	0.27AB	95 C	0.50 C	0.33B	88 C
12.0	0.50B	0.29 AB	55 D	0.60 AB	0.24C	52 D
16.0	0.44 C	0.31A	24 E	0.56 B	0.46 A	18 E

In each column, means of each season followed by the same letter are not significant at 0.05 level by Duncan' s Multiple Range Test (DMRT).

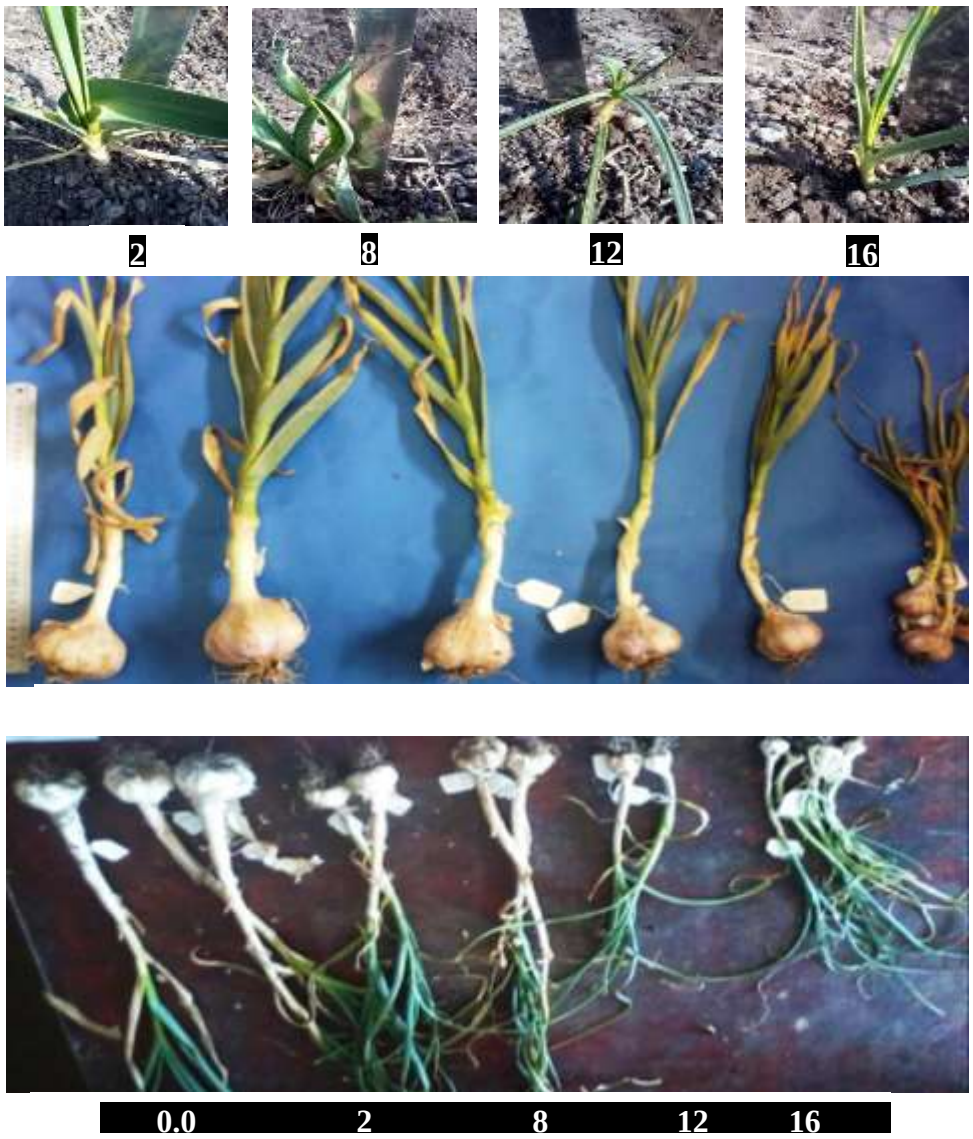

Fig 5: Some morphological changes in plants grown from cloves treated with different gamma rays.Up:60-day old plants obtained from treated cloves with 2, 8, 12, 16 gray. Middle: plants obtained from treated cloves of Egaseed 1 cultivar with 0.0, 2, 8, 12, 16, and 20 gray at harvest (180-day old plants). Bottom: plants obtained from treated cloves of White clone cultivar with 0.0, 2, 8, 12, 16, and 20 gray at harvest (180-day old plants).

REFERENCES

Ackermann, T; Mulrow, D. and Ramirez, G (2001) Garlic Shows Promise for Improving Some Cardiovascular Risk Factors Arch Intern Med 161(6):813-824.

Alverez, R.B; Delgado de la Flor, F (1996) Evaluation of population of garlic (*Allium sativum* L.) cv. Morado Arequipeno irradiation with gamma rays Rivesta di Agricultura Subtropicale e Tropicale 90:3, 369:377.

Abd El Rasheed, K. G.; Moustafa, Y. M. M.; Hassan, E. A.; Abd El Ati, Y. Y. and Gad El Hak, S. H. (2016) Traits under laboratory conditions to identify garlic genotypes suitable for organic agriculture. Egypt J. Agric. Res. 94(73-88)pp.

Agriculture Research Center (ARC) (1999) Garlic. 521/1999.

Ata, A. M (2005) Constitutive heterochromatin diversification of two *Allium* species cultivated in Egypt. Preceding of the 7th African Crop Science Society Conference, Dec.5-9, Kampala, Uganda, pp225:231.

Badr, H.M.A, El-Sahi, and Abdel-Kader, M.M (1978) Effect of low doses of gamma radiation of the growth and yield of two varieties of tomato (*Lycopersicon esculentum* L.) Alex. J. Agric. Res. 26(3): 715-720.

Batchvarov, S (1993) Garlic (*Allium sativum* L.) Genetic Improvement of vegetable crops Oxford UK. P. 15-27.

Bozzini, A (1991) Discovery of Italian fertile tetraploid lion of garlic. Econ .Bot., 45 : 436- 438.

Bradley, K. F.; Rieger, M.A. and Collins, G. G (1996) Classification of Australian garlic cultivars by DNA fingerprinting. Australian Journal of Experimental Agriculture, 36(5): 613-618.

Breslavets, L.B (1946) Plants and X-rays. Moscow: Acad. Sci. USSR Press, (Translation American Institute of Biological Sciences.

Croci,A. Arguello, J, Crvetto,A. and Orioli, G (1991) Changes in peroxidases associated with radiation – induced sprout inhibition in garlic (*Allium sativum* L.),Int.J.Radiant. Biol.59,551-557.

Datta S.K (2002) Parameters for detecting effects of ionizing radiations on plants. In: Tripathi RD Kulshreshtha K, Agarwal M, Ahmad KJ, Varshney CK, Krupa S and Pushpangadan P (eds), "Plant Responses to Environmental Stress", International Book Distributing Co., Lucknow, India: 257-265.

Datta, S.K; Debasis C; Arvind K.V; and Biresh K.B (2011) Gamma Ray induced chromosomal aberrations and enzyme related defence mechanism in *Allium cepa* L.Cariologia 64:388-397.

Datta, S.K; Hvang, Y.S; and Parra, L.C (2012) An automated method for high definition trans cranial

direct current stimulation modeling. Conf. Proc-IEE Eng-med. Bio. Soc.

Esnault M. A; Legue, F and Chenal, C *(*2010) Ionizing radiation: advances in plant response. Environmental and Experimental Botany, 68, 231–237.

Fennech M (2002) The *in-vitro* micronucleus technique. Mutation Research. 45: 81-95.

Focea, R; Capraru, G; Racuciu, M; Creanga, D and Luchian, T (2012) Aberrant cell divisions in root meristeme of maize following exposure to X-rays low doses compared to similar effects of 50 Hz electromagnetic exposure. EPJ web of Conferences 24, 06004.

Gadel-Hak, S. H. Ahmed K. Z, Mostafa, Y.M. and Asmaa S. E (2011) Growth and cytogenetical properties of micro-propagated and successfully acclimatized garlic (*Allium sativum* L.) clones with a modified shoot tip culture protocol. Journal of Horticultural Science & Ornamental Plants 3 (2): 115-129, 2011.

Gomez, K.A. and Gomez, A.A (1984) Statistical procedures for agriculture research. John Wiley& Sons New York pp.680.

Gonzalez R.E; V.C. Soto and Sance M.M.(2012) Variability of solids, organosulfur compounds, pungency and health-enhancing traits in garlic (Allium sativum L.) cultivars belonging to different ecophysiological groups. J Agric Food Chem57:10282-8.

Grant W.F (1978) Chromosome aberrations in plants as a monitoring system. Environment Health Perspectives.27: 37-43.

Hammad,A.H.A , Abd El-Halem.A.K ,Orabi, O.A and Hussein, M.M (1988) Effect of Gamma – irradiation and salinity in growth ,yield and its components and chemical composition of Barly .J.Agron.Egypt.13 (1-2):101-114.

Hassan, H.A; Wang, H; and Xixiang, D.Y (2015) Sprout differentiation and mutation induced in garlic (*Allium sativum* L.) callus exposed to gamma radiation. Plant Growth Reegul 75:465-471.

Hemada, A.A. Ahmed, S.I. and Mohamed, A.G (2012) Use of gamma ray and chemical mutagens to induce genetic variations in two garlic cultivars J. Plant production, Mansoura univ., Vol.3(11)2679:2698.

Iglesias - Enriquez, I. Rubio Cabello, J. and Danes, R (2001) Study of transportation of onion and garlic imported from Chile, irradiation and without irradiation .Alimentaria 38:325,79-83.

Ipek, M,A and Simon, P. W (2008) Genetic Characterization of *Allium tunceliannum* :An endemic edible Allium species with garlic odor. Sci Hort. 115(4):409-415.

Kaushik , G.C (1996) Cytotoxicity of cement kiln dust on mit0sis of roof tip cells in *Vicia faba.* J.

Ecotoxico Environ Monit, (1, 53-57).

Kik C , R and Gebhardt R (2001) Garlic and Health Plant Research International,
Wageningen University & Research Center, P.O. Box 16, 6700 AA Journal Article, Review, Research Support, Non-U.S. Gov't.

Koristov,YN. And Narimanov, A.A (1997) Low doses of ionizing radiation and hydrogen peroxidase stimulate plant growth. Biologia (Bratislava).52.121-124.

Kumar, N and Tiwari, R.S (1998) Effect of gamma rays irradiation on yield and yield contributing characters of garlic Allium sativum L. genotypes. .J. of bio. and Biotech.

Kumar.N.K; Hemavathi, K.L and Jajannath, S (2015) Genotoxic effect of distillery effluent on root tip cells of *Allium sativum* L.J. of bio. and Biotech. 3 (03),pp.038-041.

Lampasona, S. G; L, Mart and Burba, J.L (2003) Genetic diversity among selected Argentinean garlic clones (*Allium sativum*L.) using AFLP (Amplified Fragment Length Polymorphism). *Euphytica*132: 115–119, 2003.

Lucky, T.D (1980) Hormesis with ionizing radiation . Boca Raton Florida :CRCPress. Mann, L. K (1957) Anatomy of the garlic bulb and factors affecting bulb development. Hilgardia , 21: 195-251.

Nagovreny, R (1998) Garlic medicinal food or nutritious medicine. J Med food. 1:13-28.

Osman, S.M ; Ata, A.M. and Gad El-Hak, S.N.H (2007) Morphological germination bolting and cytogenetical characteristics of fourteen promising garlic genotypes. Proc. Afr. Crop ASci. Conf., 8:2005-2012.

Panthee, D.R.; Regmi, K.C.; Subedi, H.N.; Bhattarai, P.P. and Dhakal, S.J (2006) Diversity analysis of garlic (*Allium sativum* L.) germpasms available in Nepal based on morphological characters. Genet. Resour. Crop Evol., 53: 205-212.

Patil, B.C and Bhat, B.I (2000) A comparative study of MH and EMS in the induction of chromosomal aberrations of lateral root meristem in Clitoria terantia L. Cycologia. 57: 259-264.

Patskevich, V.M (1961) Conference on seed irradiation prior to sowing. Sov. J. At. Energy. 10, 549 –551.

Planel, H; Solihavoup, Jp; Tixador, A. Richoilly , G. Conter, A. Crout, F.Caratero, C. and Gubin, Y.R (1987) Influence on cell proliferation of background radiation or exposure to very low chronic gamma radiation. Health Phys. 52,571-578.

Preuss, A.M and Britta, A.B (1976) A DNA –damage- induced cell cycle checkpoint in synsis. Radilogy. Genetics.164,323-334

Sagan, L.A (1987) What is Hormosis and why haven't we heard it befor? Health Phys.52. 521:525.

Sanai, S.S. and Davis, G.N (1967) Karyotype analysis of some Allium species. J. Amer . Soc. Hort. Sci., 95:102-105.

Selvaraj, N.; Natarajan, S,; and Ramaraj, B (2001) Studies on induced mutations in garlic Hort. Res. Station, Tamil Nadu Agric. Univ. Vijayanagaram (India).

Simon, P.W. and Jenderek, M.M (2003) Flowering, seed production andthe genesis of garlic breeding. Plant Breed.Rev. 23: 211-244.

Shalaby ,G.I; Nasser,A.M and Faraghaly, M.A (1983)a Effect of ENS on yield and quality of Egyption garlic Assuit J.Agric.Sci.;14(1):71:79.

Shalaby ,G.I; Nasser,A.M and Faraghaly , M.A (1983)b Effect of gamma rays on garlic Assuit J.Agric.Sci.;14(1):71:79.

Swanson, C.P; Merz, T and Young, W.J (1990) Cytogenetic the Chromosome in Division, Inheritance and Evolution. (2nd Ed). Prentice-Hall Inc.

Vaijapurkar, SG; Agrawal, D; Chaudhuri, K.R; Senwar, PK. And Bharnagar, P (2001) Gamma – irradiated onions as a biological indicator of radiation dose. Rad.Meas.33, 833-836.

الملخص العربى

إستحداث اختلافات في بعض الصفات الحقلية والسيتولوجية لإثنين من التراكيب الوراثية للثوم

أسماء صلاح عزات[1] ، ياسر محمود محمد مصطفى[1] ، قاسم زكى أحمد[2] ، سيف النصر حسين جاد الحق[1] ، محمد محمود فراج[1]

[1] قسم البساتين (خضر) – كلية الزراعة – جامعة المنيا – جمهورية مصر العربية.
[2]قسم الوراثة – كلية الزراعة – جامعة المنيا – جمهورية مصر العربية.

اجريت هذه الدراسة خلال موسمي الزراعة 2013 و 2014 تم معاملة فصوص ثوم (سلالة بيضاء وصنف ايجاسيد1) الثوم بجرعات مختلفة من أشعة جاما (2 و 8 و 12 و 16 و 20 و 24 جراي بمركز بحوث تكنولوجيا الإشعاع بهيئة الطاقة الذرية في القاهرة) وتم دراسة بعض الصفات تحت ظروف الحقل والمعمل في التجربة المعملية تم دراسة نسبة الانبات و بالتعاون مع قسم الوراثة كلية الزراعة جامعة المنيا تم دراسة السلوك السيتولوجي للنباتات المعاملة بالاشعاع بالاضافة للنباتات غير المعاملة (كنترول) وفي تجربة حقلية تم زراعة الفصوص المعاملة بالاضافة الي الكنترول في مزرعة قسم البساتين فرع الخضر بكلية الزراعة جامعة المنيا وذلك خلال الجيل الأول بعد المعاملة بالإشعاع وتم دراسة المعاملات من الناحية الحقلية عن طريق دراسة بعض الصفات الحقلية للنباتات . وأظهرت النتائج اختلافات معنوية بين المعاملات وبعضها وأن الجرعات العالية من أشعة جاما أدت الي انخفاض نسبة الإنبات وزيادة الشذوذ الكروموسومي في خلايا النباتات و اعتبرت الجرعتين 20 و 24 جراي جرعات مميتة وذلك مقارنة بالجرعات المنخفضة و الكنترول وايضاً أظهرت النتائج أن الجرعة 2 جراي اعطت زيادة معنوية في معظم الصفات الحقلية محل الدراسة وذلك في كلا التركيبين الوراثيين (السلالة البيضاء وصنف ايجاسيد 1).