Determination of Suitable Gamma-Ray Dose for Mutation Induction in Garlic (*Allium sativum* L.) and Identification of Genetic Diversity by RAPD and ISSR markers

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Ahmed M. Mahmoud[1], Shereen Abu El-Maaty [2], Neama H. Osman[2], and Khaled F. Al-Azab[3]
[1]Vegetable Crops Department, Faculty of Agriculture, Cairo University, Giza, 12613, Egypt.
[2]Genetics Department, Faculty of Agriculture, Cairo University, Giza, 12613, Egypt.
[3]Plant Research Department, Nuclear Research Center, Atomic Energy Authority, Inshas, 13759, Egypt.

ABSTRACT

As an initial step in a mutation breeding program to improve two Egyptian garlic (Allium sativum L.) clonal cvs Balady and Sids-40, this experiment was conducted to test the radio-sensitivity for these clones to different gamma-ray doses (0, 2, 4, 6, and 8 Gy) and to evaluate the genetic diversity between different doses treatments by using the molecular markers. The suitable dose that induce mutation without adverse effect on germination and plant growth was determined using 50% plant mortality (LD_{50}) based on the probit analysis. RAPD and ISSR markers were used to evaluate the genetic diversity. Increasing gamma-ray dose led to a decrease in the germination percentage and an increase in the mortality percentage. The inhibitory effects of high doses (6 and 8 Gy) and the stimulant effects of low doses (2 and 4 Gy) were clearly shown on the plant growth and the formed bulb traits. The LD_{50} was 6.42 Gy for 'Balady' and 6.93 Gy for 'Sids-40'. ISSR markers revealed a higher polymorphism (100 %) than did RAPD (68.6 %). The genetic similarity coefficient ranged from 0.5812 to 0.87591. Moreover, the constructed dendrogram classified Sids-40 and Balady with 6 Gy into one cluster and all the other treatments and Balady were grouped into another cluster, thus indicating higher genetic distinctness among different treatments of gamma rays. Accordingly, this study suggested the possibility of using the probit analysis to determine the appropriate dose of gamma irradiation, as well as using ISSR and RAPD markers to determine the genetic diversity in garlic.

Key words: *Allium sativum, LD_{50}, Probit analysis, RAPD, ISSR.*

INTRODUCTION

Widely consumed for food and medicine purposes, Garlic (*Allium sativum* L.) is an important obligate apomictic plant that is vegetatively propagated by cloves and bulbils where there are neither flowers nor true seeds. This characteristic has led to a lack of genetic variation and cultivars number. Hence, there is an urgent need for garlic improvement, to increase its productivity and enhance its quality and disease resistance traits. One of the ways of ensuring genetic variation is through mutation (Ezzat *et al*., 2016; Hemada *et al*., 2012; Mostafa *et al*., 2015). Al-Safadi *et al*. (2000) and Metwally and Abou Shousha (2002) have suggested garlic improvement through mutation.

The most essential aspect of mutation breeding is selecting the suitable mutagenic agent (mutagen) and dose and developing a method to determine the mutants. The most widely used effective mutagens are gamma (γ) and X-rays. Gamma ray is considered as an ionizing radiation obtained mainly from radioactive isotopes with cobalt 60 (60Co) and cesium 137 (137Cs) as the main sources of radiation (1982). High γ-irradiation doses are more effective in mutation induction. However, higher doses of γ-irradiation inhibit garlic clove germination and cell mitosis divisions as a result of nuclear aberrations (Ezzat *et al*., 2016). Seed germination might be seen in high-dose practices; nevertheless, such method was associated with a slow growth rate and plant mortality (Hemada *et al*., 2012 and Pellegrini *et al*., 2000). By contrast, low doses of γ rays stimulate cell divisions, growth, and development of garlic plants (Ezzat *et al*., 2016; Hemada *et al*., 2012 and Mostafa *et al*., 2015). Therefore, before its large-scale applications, the appropriate dose of the mutagen should be determined to avoid excessive loss of experimental materials. As a starting point, radio-sensitivity tests must be conducted to determine the lethal dosage value (LD50), which in turn is used to define the exact mutation dose. The literature is replete with many studies that determined a suitable mutagen dose for garlic (Mostafa *et al*., 2015; Gharib *et al*., 2006 and Taner *et al*., 2004). The findings of these studies are different. Where, garlic sensitivity to irradiation varies according to the cultivar (Mostafa *et al*., 2015), garlic clove size (Pérez-Talavera *et al*. 1999), and the state of the dormant or non-dormant garlic cloves (Pellegrini *et al*., 2001). Also, there are no accurate methods to determine the suitable dose. Most of these studies has determined the suitable dose according to the high dose, which killed the plants.

Garlic germplasm has been characterized mainly based on phenotypic characteristics. However, morphological characteristics can vary under different agroclimatic conditions (Al-Zahim *et al*., 1999). DNA polymorphisms are the choice markers for identifying and characterizing plants. They may represent the whole genome and they are not subject to environmental modification (Chen *et al*., 2014). Random amplified polymorphic DNA (RAPD) (Nabulsi *et al*., 2001; Ipek *et al*., 2003 and Ali *et al*., 2015) and inter simple sequence repeat (ISSR) (Chen *et al*., 2014 and Jabbes *et al*., 2011) markers have been used to assess genetic diversity (GD) and the relationships between and among garlic clones.

Therefore, to determine the appropriate gamma-ray dose that will induce mutations in the two local garlic clonal cvs Balady and Sids-40 without any negative effects on germination and plant growth, this study aims to achieve the following objectives: 1) to determine the impact of different gamma-ray doses on germination, growth, productivity, and storability under natural conditions and to determine the lethal dose (LD50) using the probit analysis; and 2) to evaluate the genetic variation among the clones and the irritated treatments using RAPD and ISSR markers.

MATERIALS AND METHODS

This study was conducted at the Agricultural Experiment Station of the Faculty of Agriculture, Cairo University, Giza, Egypt, during the 2015/2016 and 2016/2017 winter seasons. Bulbs of the two garlic cvs were obtained from the Sids Research Station, Agricultural Research Center, Beni-Suef Governorate, Egypt.

Procedure of radiation and planting garlic cloves

On the first of September of both seasons and 24 h before planting, 100 semi-dry large cloves of both cvs per treatment were packed in plastic-bags and irradiated with 137Cs as a γ-radiation source using different doses (0, 2, 4, 6, and 8 Gy), with a dose rate of 0.713 rad/sec at the National Center for Radiation Research and Technology, Nasr City, Cairo, Egypt. The irradiated cloves were directly soaked in distilled water for 24 h under room temperature. For one month under siren-house conditions, the soaked cloves were germinated in Styrofoam trays (209 cell/tray with tray dimensions of 65 cm × 38 cm × 8.3 cm) filled with a mixture of peatmoss and vermiculate (volume 1:1). The germinated cloves were field-transplanted under drip irrigation system on ridges 40 cm width × 18 m length with one drip line (30 cm between drips) and with 20 cm between plants on both sides of the drip line. The irrigation, fertilization, weed control and diseases control for the garlic cultivation were as recommended. The cloves and germinated cloves of different treatments were arranged in a randomized complete block design (RCBD) with four replicates.

Effects of radiation on germination and mortality percentages and determination of lethal dose (LD_{50})

After one month of planting cloves, the germinated cloves that have one green leaf blade were counted and the germination percentage (GP) was calculated using this formula: GP (%) = (Total No of germinated cloves/ Total No of cloves) × 100.

Before harvesting the formed bulbs, the mortality percentage (MP) was calculated based on the number of dead plants and abnormal growing plants using the formula; MP = [(Total No of un-germinated cloves + Total No of dead and abnormal plants)/Total No of cloves] × 100. To calculate the lethal dose (50% - LD50), a probit analysis was done using the MP of treated cloves to that of control cloves (Finney, 1978) as described by Ramchander *et al.* (2015).

Effects of gamma radiation doses on plant growth, bulb traits and storability of garlic

In mid-April in both seasons, the following readings were recorded on growing plants to show the phenotypic changes of each cv.: the plant length (cm) and No of leaves/plant. Irrigation was stopped for 15 days, and the plants were harvested at the end of April. Next, the bulbs were left in a well-ventilated shaded place for curing. The following traits were estimated on 10 cured bulbs/experimental unit: the equatorial and polar diameters (mm), average weights of bulb and clove (g), No of cloves/bulb, total soluble solids (TSS %) and dry matter ratio of cloves.

The cured bulbs were stored four months under room temperature conditions from May to the first of September and the weight loss was recorded at fixed intervals (1, 2, 3 and 4 months of storage).

Using RAPD and ISSR markers for genetic diversity evaluation among several radiation doses

The total genomic DNA was isolated from young leaves of normal garlic germinated plants from treated and untreated cloves using the CTAB method (Murray and Thompson, 1980). Ten primers of each RAPD and ISSR (Table 1) obtained from Bio Basic Canada Inc. were used. PCR amplification were performed in 20 µL reaction volume containing 1 µ genomic DNA (20 ng/µL), 10 µL master mix (Biotecke Corporation), 1 µL primer (100 ng/µL) and 8 µL of nuclease free water. The amplification conditions were programmed with an initial step of 5 min at 94°C. After that, the amplification reaction was carried out using 40 cycles of 1 min at 94°C for denaturation, an annealing step of 1 min at different temperature degrees according to primer (Table 1), an elongation step of 1 min at 72°C and finally a terminal extension cycle at 72°C for 5 min. The PCR products were resolved with 1.5% agarose gel in 1x TAE buffer, DNA bands visualized with ethidium bromide staining (0.5 µg/ml) and photographed under UV light using the gel documentation system (Bio-Rad® Gel Doc-2000). One kb ladder DNA was used as the molecular weight size marker.

Statistical analysis

Results of the two seasons were combined and statistically analyzed using MSTAT-C v. 2.1 (Michigan State University, Michigan, USA) and the mean comparisons were based on the LSD5% test (Steel and Torrie, 1981). The probit analysis software log dose probit (Ldp Line software, copyright 2000 by Ehab Mostafa Bakr, Cairo, Egypt) was used to calculate the LD50 (www.ehabsoft.com/ldpline). Clear bands in the gel profiles of RAPD and ISSR's were recorded as present (1) and absent (0). The genetic similarity coefficients were estimated according to the Dice coefficient (Sneath and Sokal, 1973). The dendrogram based on unweighted pair group method with arithmetic mean algorithm (UPGMA) was generated by using the computer program systat ver. 7 (SPSS Inc. 1997 SPSS Inc.3/9`7 standard version) (Yang and Quiros, 1993).

Table (1): The nucleotide sequence of the RAPD and ISSR primers used for PCR analysis.

Primer	Sequence (5`-3`)	Annealing Temperature
I. RAPD primers		
OPA-13	CAGCACCCAC	34
OPA-14	TCTGTGCTGG	32
OPB-05	TGCGCCCTTC	34
OPB-06	TGCTCTGCCC	34
OPB-14	TCCGCTCTGG	34
OPC-15	GACGGATCAG	32
AB-17	TCGCATCCAG	37
AC-20	ACGGAAGTGG	37
OPAR-11	GGGAAGACGG	34
OPAR-15	ACACTCTGCC	32
II. ISSR primers		
808	(AG)8C	54
810	(AG)8T	52
811	(GA)8C	54
817	(CA)8A	52
841	(GA)8YC	54
17898A	(CA)6AC	54
17898B	(CA)6GT	54
17899B	(CA)6GG	54
UBC824	(TC)8G	54
UBC826	(AC)8C	54

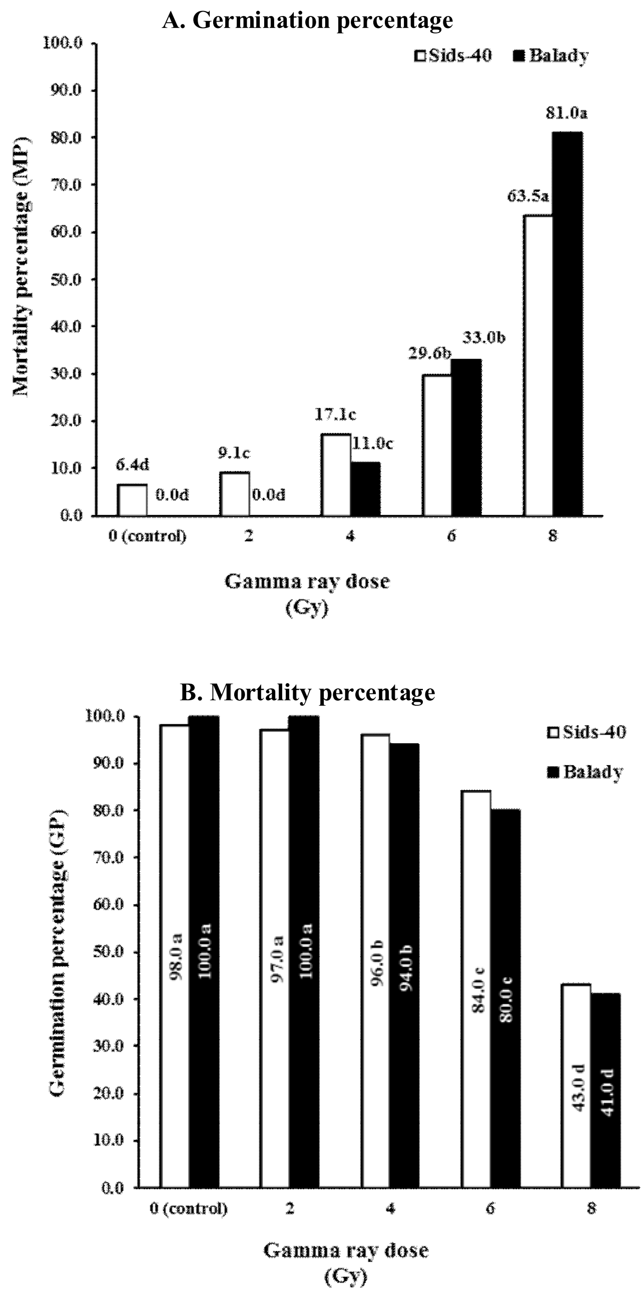

Fig. (1): Effect of different gamma ray doses on germination (A) and mortality (B) percentages of garlic cvs 'Sids-40' and 'Balady'.

RESULTS

Effects of gamma radiation on cloves germination and plant mortality

The increase in the radiation dose was inhibited clove germination (Fig. 1A). The high γ ray treatment (8 Gy) had the lowest significant GP for both cvs (43 and 41%, respectively), while, the low γ ray treatment (2 Gy) had the highest significant GP for both cvs (97 and 100 %, respectively) without significant differences from the control. Furthermore, damages of γ-ray on grown plants were observed by estimating the MP (Fig. 1B). The high dose treatment (8 Gy) had the highest significant MP for both cvs (63.5 and 81 %, respectively) compared to control. The low γ dose treatment (2 Gy) had the lowest significant MP for both cvs (9.1 and 0 %, respectively) without significant differences from the control of only Balady cultivar.

Determination of gamma ray LD_{50} for garlic

According to the results of a probit analysis shown in Fig. 2, the LD50 for cv. Sids-40 was 6.93 Gy and that for cv. Balady was 6.42 Gy. The $\chi 2$ test revealed that the used population for each cv. in this study was heterogeneous.

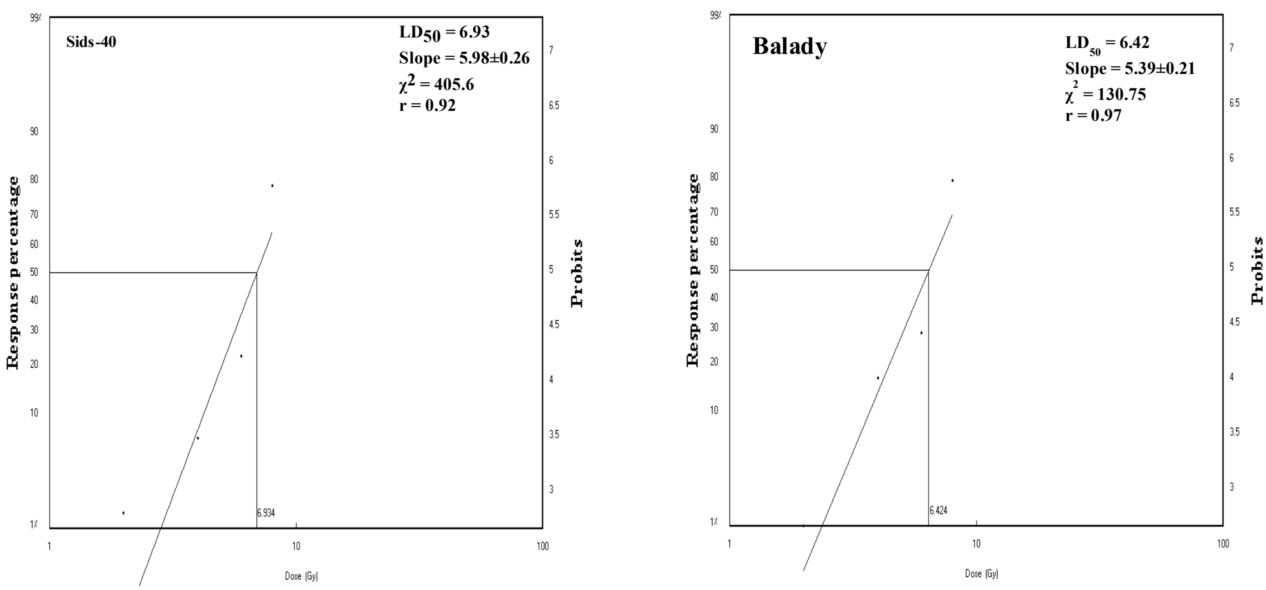

Fig. (2): Graphical representation of mortality percentage and LD_{50} for radiation doses on garlic 'Sids-40' and 'Balady' by Ldp line software.

Effect of gamma radiation on plant growth, bulb traits, and storability

The influence of γ ray doses on phenotypic changes of garlic plants was studied by measuring the biometrical plant traits length and No of leaves/plant (Fig. 3) and the formed bulb traits (Table 2). Data showed the significant effect of radiation doses on the phenotypes of plant length and No of leaves/plant traits of both cvs. Low doses of γ-radiation had a relative stimulating effect on plant growth (Fig. 3A and B).

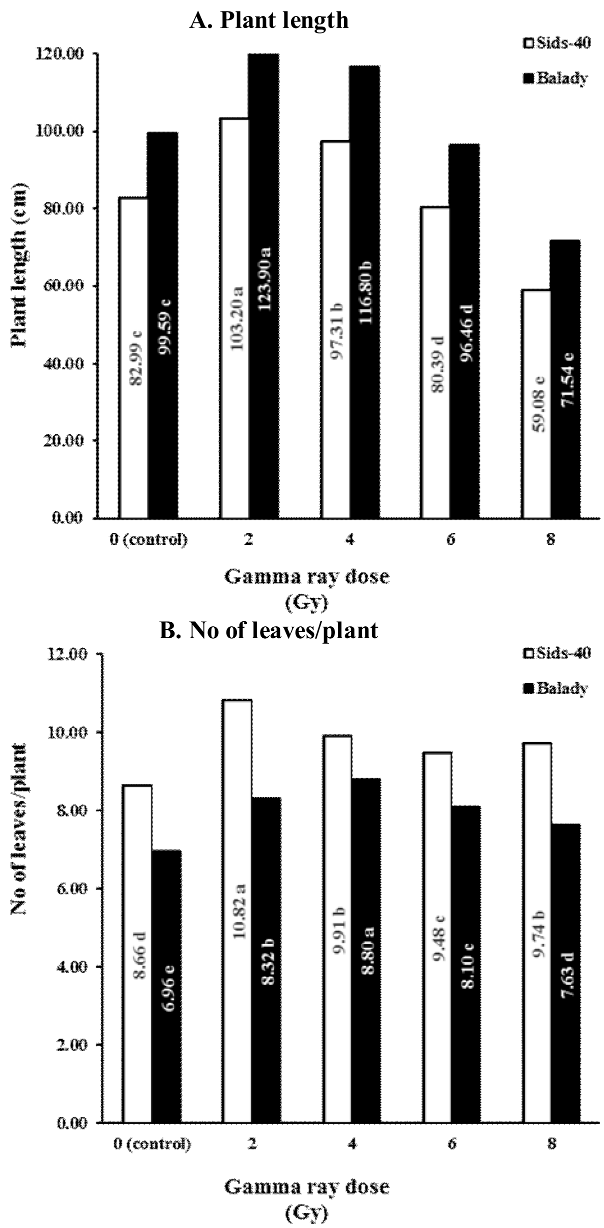

Fig. (3): Gamma-radiation effects on plant length (A) and No of leaves/plant (B) of garlic cvs 'Sids-40' and 'Balady'.

As for the traits of equatorial and polar diameters (Table 2), the 2 Gy dose gave the largest significant equatorial diameter for bulbs of both cvs (55.4 and 45.5mm, respectively), followed by 4 Gy dose with Sids-40 cultivar without significant differences. For Balady cultivar, the control treatment produced the lowest equatorial diameter (34.6 mm) followed by the high γ doses with significant differences. Nonetheless, the high γ doses provided the lowest bulb equatorial diameter for 'Sids-40'. In the same trend, readings for polar diameter trait showed that 2 Gy dose gave higher bulb polar diameter of both cvs without significant differences with the control treatment in only 'Sids-40' (43.3 and 43.1 mm, respectively).

The increase in the radiation dose reduced average bulb weight (ABW) of both cvs (Table 2). The biggest ABW of both cvs was accompanied by 2 Gy dose (54.3 and 24.4 g, respectively), while the high γ dose (8 Gy) produced the lowest significant ABW of both cvs (20.05 and 14.3 g, respectively). The No of cloves/bulb (CN/B) was increased when the radiation dose was high compared to the control in both cvs. Sids-40 cultivar gave the largest CN/B with 6 and 8 Gy doses (21.7 and 21.4, respectively), whereas, Balady cultivar provided the largest CN/B with 4 Gy dose (44.2). Also, synchronizing with the highest ABW with 2 Gy dose indicated that the highest average clove weight (ACW) was accompanied by 2 Gy dose for both cvs (2.8 and 0.6 g, respectively) compared to the other doses and the control. In addition, TSS% of bulb was increased by increasing the radiation dose compared to the control. Bulbs of 8 Gy dose had the highest TSS% in both cvs (35.9 and 33.9%, respectively) followed by bulbs of 2 Gy dose (35.4%) without significant differences for Sids-40 garlic. As for dry matter percentage (DM %), bulbs of doses 6 Gy and 8 Gy yielded higher DM% for both cvs and bulbs of 4 Gy dose for Balady garlic. However, the control bulbs were lower in DM% for both cvs. Garlic storage abilities under natural conditions during the hot months (from May to September), when there were a high respiration rate and a loss of dry matter, was estimated by determining the loss in bulb weight (Table 3).

The 6 Gy dose was the most effective treatment for reducing the weight loss percentage (WLP) with Sids-40 garlic during the first three months of the storage period (7.4, 7.1, 11.7 %, respectively), followed by 8 Gy dose (9.5, 6.9, and 10.6 %, respectively) with no significant differences only during the second and third months of storage. In the fourth month of storage, 8 Gy dose reduced the WLP (19.1 %) followed by 6 Gy dose (23.7 %) without significant differences. For Balady garlic, radiation doses had the same effect as did Sids-40 garlic during the first three storage months. In the fourth month, 8 Gy dose had a significant effect in terms of reducing WLP of both cvs (19.1 and 19.5 %, respectively) from other radiation doses.

Detection of genetic diversity by RAPD and ISSR markers

Ten random primers generated reproducible RAPD profiles (Fig. 4) with a number of amplified DNA fragments ranging from 6 to 10 amplicons per primer (Table 4). The total number of fragments produced by the primers were 83 with an average of 8.3 amplicons per primer. The size of amplicons varied with different primers, ranging from 143 to 4766 base pairs. Primer OPAR-15 amplified the highest number of amplicons (10) with a polymorphism of 90 %. This primer also recorded a unique fragment at molecular weight of 103 bp which was specific to treated Sids-40 with 8Gy. This result indicated, the effect of γ-ray dose, followed by OPB-06 which generated 10 amplicons with polymorphism of 60%. The primer OPAR-11 showed the minimum numbers of amplicons (6) with only one monomorphic band. On average, RAPD primers revealed 68.6% total polymorphisms among garlic controls and treatments.

Table (2): Combined data across seasons 2016 and 2017 of γ radiation dose effects on bulb traits of garlic cvs 'Sids-40' and 'Balady'.

Cultivar	Gamma dose (Gy)	Bulb equatorial diameter (mm)	Bulb polar diameter (mm)	Average Bulb weight (g)	Number of cloves /bulb	Average clove weight (g)	TSS (%)	Dry matter[z] (%)
'Sids-40'	0 (control)	51.40 b	43.08 a	34.12 c	13.93 d	2.36 b	32.53 d	39.69 d
	2	54.50 a	43.39 a	54.26 a	18.94 c	2.79 a	35.40 ab	41.28 b
	4	55.40 a	40.65 b	40.45 b	20.21 b	1.97 c	34.15 c	40.88 c
	6	48.96 c	40.76 b	26.28 d	21.70 a	1.19 d	35.16 b	41.65 a
	8	48.19 c	39.16 c	20.05 e	21.38 a	0.96 e	35.86 a	41.79 a
'Balady"	0 (control)	34.57 d	26.48 d	14.19 d	32.53 d	0.44 c	30.08 d	40.15 c
	2	45.53 a	33.40 a	24.38 a	41.75 b	0.60 a	30.85 c	41.50 b
	4	41.83 b	32.26 b	22.06 b	44.19 a	0.50 b	31.01 c	43.21 a
	6	40.41 bc	29.10 c	19.60 c	40.06 c	0.51 b	32.55 b	42.80 a
	8	39.65 c	28.69 c	14.32 d	38.79 c	0.38 d	33.94 a	42.76 a

[z]The data in percentages were transformed by arc-sin formula.

Table (3): Effect of γ doses on weight loss percentage[z] (WLP) of garlic cvs Sids-40 and Balady during the storage periods.

Cultivar	Gamma Dose (Gy)	WLP after 1 month (%)	WLP after 2 month (%)	WLP after 3 month (%)	WLP after 4 month (%)
'Sids-40'	0 (control)	16.11 a	13.74 a	23.35 a	24.15 a
	2	15.86 ab	12.52 b	21.59 a	24.18 a
	4	13.89 b	10.27 c	19.30 b	22.69 ab
	6	7.35 d	7.09 d	11.69 c	23.67 ab
	8	9.52 c	6.94 d	10.59 c	19.14 b
'Balady'	0 (control)	14.90 b	14.19 a	22.78 a	19.82 a
	2	17.89 a	13.35 b	21.44 a	20.93 a
	4	13.47 b	10.37 c	17.49 b	16.56 b
	6	5.98 d	6.40 d	9.07 c	14.87 b
	8	8.39 c	6.60 d	7.98 c	19.46 c

[z]The data in percentages were transformed by arc-sin formula.

The ISSR primers revealed 67 amplicons with an average of 6.7 bands per primer (Table 4 and Fig. 5). The molecular weight of the different bands ranged from 200 to 2300 bp. The ISSR primers 808, 810, 811, 17898A and UBC824 exhibited the highest polymorphism 100 % among controls and treatments followed by primer 841 which showed 85.7 % polymorphisms. On the other hand, the primer 17898B generated the minimum polymorphism (28.5 %) with five common monomorphic bands. The Primer 17899B was showed three unique markers at a molecular weight of 625, 330 and 250 specific for the treated 'Balady' with 6 Gy followed by primer 17898A which revealed two unique markers specific for the treated 'Balady' with 8 Gy at 1500 and 1400 bp.

The level of GS value ranged from 0.5812 to 0.87591. The highest GS (0.87591) was observed between treatments of Sids-40 with 2 Gy and 6 Gy followed by treatments of Sids-40 with 4 Gy and 6 Gy (0.87210). Meanwhile, the lowest GS (0.5812) was observed between treated Sids-40 with 8 Gy and treated Balady with 6 Gy. The UPGMA clustering dendrogram based on the Dice similarity index was obtained (Table 5 and Fig. 6). The RAPD and ISSR data clustered the controls of Sids-40 and Balady with 6 Gy in one cluster. On the other hand, all the treatments and control of Balady grouped in the second cluster were divided into two subclusters. The first one contained treatments of Balady with 2 G, 4, and 8 Gy together. The other subcluster was subdivided into two groups; the first included the treated 'Sids-40' with only 8 Gy. The second group contained treatments of Sids-40 with 2, 4, and 6 Gy, including the control of Balady.

Table (4): Total number of amplicons, monomorphic amplicons, polymorphic amplicons and percentage of polymorphism as revealed by RAPD and ISSR markers among the garlic controls and radiation treatments.

Name of primer	Total No. of amplicons	Monomorphic amplicons	Polymorphic amplicons	Percent of polymorphism
I. RAPD				
OPA-13	9	3	6	66.6
OPA-14	9	2	7	77.7
OPB-05	9	5	4	44.4
OPB-06	10	4	6	60.0
OPB-14	8	4	4	50.0
OPC-15	7	2	5	71.4
AB-17	8	2	6	75.0
AC-20	7	2	5	71.4
OPAR-11	6	1	5	83.3
OPAR-15	10	1	9	90.0
Total	83	26	57	-
Average	8.3	2.6	5.7	68.6
II. ISSR				
808	7	-	7	100
810	6	-	6	100
811	7	-	7	100
817	6	2	4	66.6
841	7	1	6	85.7
17898A	5	-	5	100
17898B	7	5	2	28.5
17899B	10	4	6	60
UBC824	8	-	8	100
UBC826	4	2	2	50
Total	67	14	53	-
Average	6.7	1.4	5.3	7.9

DISCUSSION

The biological effect of γ rays based on the interaction with atoms or molecules in the cell, particularly with water to produce free radicals. These radicals can damage or modify important components of plant cells, and they have been reported to differentially affect the morphology, anatomy, biochemistry and physiology of plants depending on the irradiation level (Kovács and Keresztes, 2002 and Wi *et al*., 2007). Several factors profoundly influenced radiation effects. Some related to plant characteristics such as species, cultivar, stage of development, tissue architecture and genome organization; and some related to radiation features such as the quality, dose, and duration of exposure (Jan *et al*., 2012 and Hassan *et al*., 2015). Nevertheless, a radio-sensitivity test was conducted at the beginning of the mutation breeding program to determine the appropriate dose. Several reports regarding the use of γ rays to induce mutations in garlic about in the literature, but the results were different in terms of appropriate doses (Mostafa *et al*., 2015; Choudhary and Dnyansagar, 1982; Pérez-Talavera and Cervantes, 1999 and Pellegrini *et al*., 2001). Al-Safadi *et al*. (2000), Nabulsi *et al*. (2001), Taner *et al*. (2004) and Hassan *et al*. (2015) found that optimal doses of γ rays that induce mutation in A. sativum varied from 1 to 7 Gy. However, Gharib *et al*. (2006) and Roldán (2015) found that for irradiated calli, an optimal dose of γ rays ranged from 5 to 10 Gy.

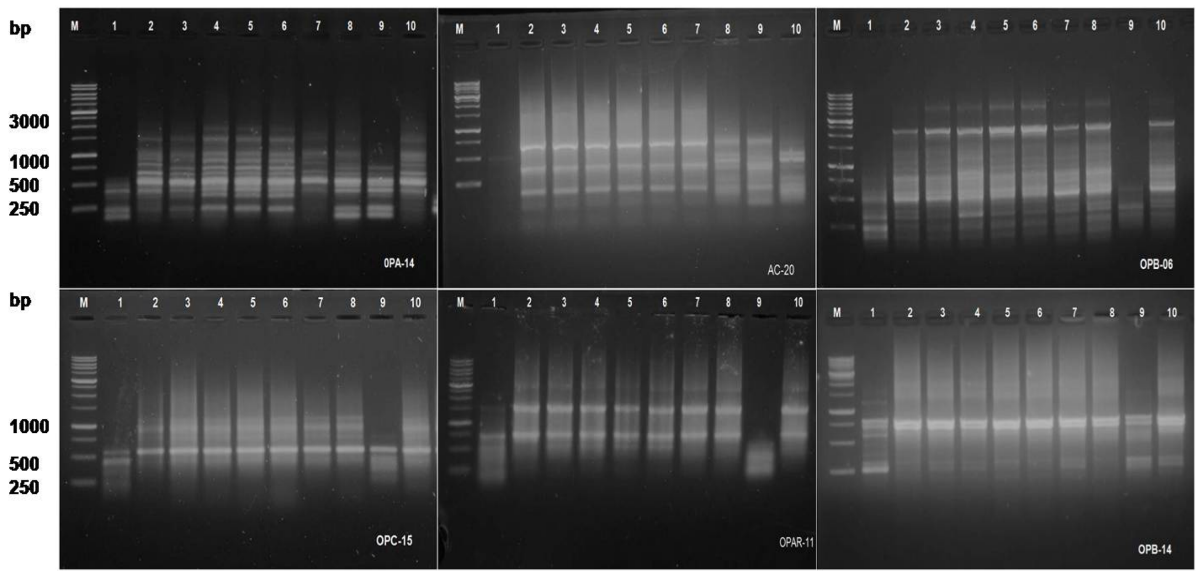

Fig. (4):. RAPD profiles of the garlic genotypes amplified with RAPD primers. M, molecular weight marker (1 Kb DNA ladder); 1, Sids-40; 2, Balady; 3, Sids-40 with 2Gy; 4, Sids-40 with 4 Gy; 5, Sids-40 with 6 Gy; 6, Sids-40 with 8 Gy; 7, Balady with 2 Gy; 8, Balady with 4 Gy; 9, Balady with 6 Gy; and 10, Balady with 8 Gy.

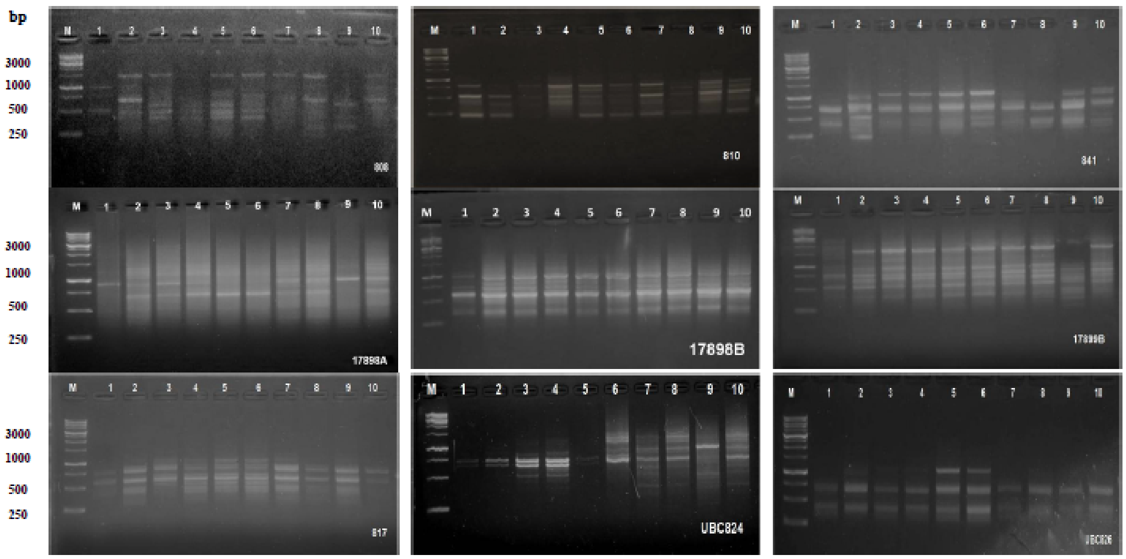

Fig. (5): ISSR profiles of the garlic genotypes amplified with ISSR primers. M, molecular weight marker (1 Kb DNA ladder); 1, Sids-40; 2, Balady; 3, Sids-40 with 2Gy; 4, Sids-40 with 4 Gy; 5, Sids-40 with 6 Gy; 6, Sids-40 with 8 Gy; 7, Balady with 2 Gy; 8, Balady with 4 Gy; 9, Balady with 6 Gy; and 10, Balady with 8 Gy.

Table (5): Genetic similarity (GS) matrices computed according to Dice coefficient from RAPD and ISSR combined data.

	Sids-40	Balady	Sids-40 with 2 Gy	Sids-40 with 4 Gy	Sids-40 with 6 Gy	Sids-40 with 8 Gy	Balady with 2 Gy	Balady with 4 Gy	Balady with 6 Gy	Balady with 8 Gy
Sids-40	1									
Balady	0.67273	1								
Sids-40 with 2 Gy	0.66055	0.85926	1							
Sids-40 with 4 Gy	0.66667	0.83969	0.86154	1						
Sids-40 with 6 Gy	0.62500	0.86957	0.87591	0.87218	1					
Sids-40 with 8 Gy	0.62385	0.80000	0.85075	0.83077	0.86131	1				
Balady with 2 Gy	0.63063	0.83212	0.83824	0.83333	0.83453	0.80882	1			
Balady with 4 Gy	0.61818	0.85294	0.80000	0.74809	0.78261	0.78519	0.86131	1		
Balady with 6 Gy	0.67391	0.62712	0.59829	0.65487	0.61667	0.58120	0.63866	0.66102	1	
Balady with 8 Gy	0.66087	0.80851	0.78571	0.80882	0.79720	0.78571	0.83099	0.85106	0.65041	1

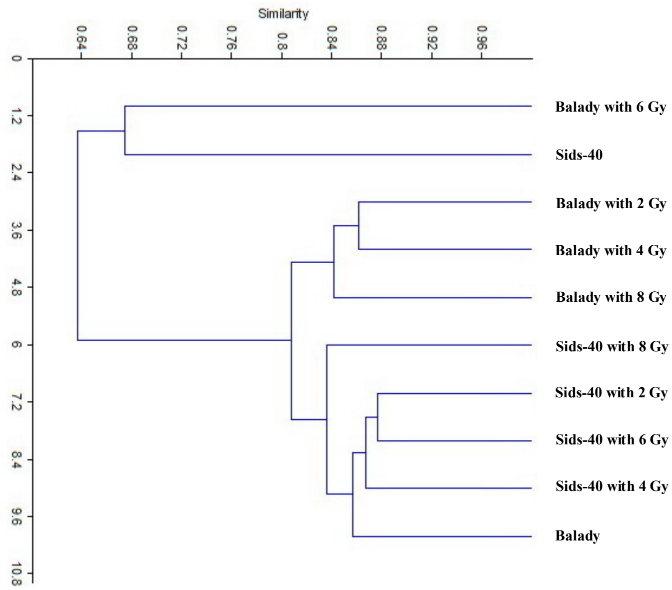

Fig. (6): Dendrogram for the garlic controls and radiation treatments constructed from RAPD and ISSR data using Unweighted Pair-Group Arithmetic Average (UPGMA) and similarity matrices computed according to Dice coefficients.

Generally, the high doses of γ ray inhibited garlic cloves germination and plant growth. This effect was reflected in the formed bulb traits. Pellegrini *et al*. (2000) and Ezzat *et al*. (2016) reported that high γ doses (> 10 and > 16 Gy, respectively) inhibited germination in garlic. The inhibition of cloves germination at higher γ doses might be attributed to numerous histological and cytological changes (Ezzat *et al*., 2016 and Ali *et al*., 2015), the alteration of enzyme activities which disturb the physiological and biological processes in the cell (Hassan *et al*., 2015), and the impaired mitosis or virtual elimination of cell division in the meristematic zones during germination (Ali *et al*., 2015). Clove germination in radiation treatments, does not mean that all germinated cloves can complete their growth naturally; some of them will die or grow abnormally (Hemada *et al*., 2012). Therefore, MP results were increased with more gamma-ray doses. Pérez *et al*. (2007) found that the inhibition of sprout growth in garlic was due to a reduction in phospholipids, glycolipids, and neutral lipids after γ-irradiation. Wi *et al*. (2007) mentioned that high radiation doses could affect cells, tissues and organelles such as chloroplast and that they could inhibit cell division processes, multiplication and growth or development of tissues.

Radiation treatments reduced the weight loss of bulbs during the storage periods, especially with high doses, which are characterized by high content of dry matter (Table 2) and low bulb weight and size (low surface exposed to respiration). They can also reduce the respiration rate and loss of dry matter. These results are consistent with those of Pellegrini *et al*. (2000), who found that γ-radiation reduced monthly weight loss of garlic bulbs. Al-Safadi *et al*. (2000) improved storability of garlic *via* mutations, with one or more genes controlling this character.

The effect of gamma irradiation on the plant phenotypic variation was observed by estimating morphological characteristics of plants. However, the variance in morphological characteristics may be due to the environmental differences. Therefore, the genetic diversity between radiation treatments and the control (untreated) was estimated based on DNA markers RAPD and ISSR and by estimating GS. ISSR revealed a higher polymorphism among garlic treatments than did RAPD. What is more, the genetic similarity results indicated that treatments of "Sids-40" with 4 and 6 Gy were distantly related, and these doses had the same effect. This includes the high genetic diversity between treatments Sids-40 with 8 Gy and Balady with 6 Gy. Results of the UPGMA clustering dendrogram revealed the highest genetic relationship because of the effect of γ rays. Besides, the genetic background of "Sids-40" genotype was different from all genotypes due to the effect of γ ray (8 Gy), and the high genetic distinctness between treatments Sids-40 with 2, 4 and 6 Gy from the other treatments of radiation.

CONCLUSION

Regarding the results of this study, the appropriate doses (LD50) of γ radiation that cause high mutation frequency without affecting the vitality and plant growth was 6.93 Gy for Sids-40 garlic and 6.42 Gy for Balady garlic. The genetic relationship of the morphological variations can be determined by using RAPD and ISSR analyses.

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الملخص العربي

تحديد الجرعة المناسبة من أشعة جاما الحاثة على الطفور فى الثوم (*Allium sativum* L.) و تمييز التباين الوراثى بواسطة المعلمات RAPD و ISSR

أحمد محمد على محمود[1]، و شيرين أبوالمعاطى[2]، و نعمه حسين عثمان[2]، و خالد فؤاد العزب[3]
[1]قسم الخضر - كلية الزراعة، جامعة القاهرة
[2]قسم الوراثة - كلية الزراعة، جامعة القاهرة
[3]قسم بحوث النبات، مركز البحوث النووية، هيئة الطاقة الذرية - إنشاص، مصر

كخطوة أولية فى برنامج تربية لتحسين صنفى الثوم (*Allium sativum* L.) "البلدى" و "سدس-٤٠"، أجريت تلك التجربة لإختبار الحساسية الإشعاعية لتلك الأصناف لجرعات مختلفة من أشعة جاما (صفر و ٢ و ٤ و ٦ و ٨ جراى) و تقييم التنوع الوراثى بين المعاملات المختلفة باستخدام المعلمات الجزيئية. الجرعة المناسبة الحاثة على الطفور بدون تأثير ضار على الإنبات و نمو النبات كانت حددت باستخدام الجرعة الحادثة لموت ٥٠% من النباتات (LD_{50}) إعتماداً على تحليل الـ Probit analysis. كذلك، استخدمت المعلمات الجزيئية RAPD و ISSR لتقييم التنوع الوراثى. الزيادة فى جرعة جاما أدت لنقص نسبة الإنبات و زيادة نسبة الموت. التأثيرات المثبطة للجرعات العالية (٦ و ٨ جراى) و التأثيرات المحفزة للجرعات المنخفضة (٢ و ٤ جراى) ظهرت بوضوح على نمو النبات و صفات الأبصال المتكونة. الجرعة المميتة LD50 كانت للصنف البلدى ٦,٤٢ جراى و كانت للصنف سدس-٤٠ ٦,٩٣. المعلمات الجزيئية ISSR اظهرت نسبة تباين مظهرى ١٠٠% أعلى من المعلمات الجزيئية RAPD (٦٨.٨%). نسبة التشابه الوراثى ترواح بين ٠,٥٨١٢ لـ ٠,٨٧٥٩١. علاوة على ذلك، الـ Constructed dendrogram وضع الصنف سدس-٤٠ و الصنف البلدى المعامل بـ ٦ جراى فى عنقود واحد و باقى المعاملات و الصنف البلدى فى عنقود أخر، ذلك يشير إلى التباعد الوراثى بين المعاملات المختلفة لاشعة جاما.وفقاً لذلك، تلك الدراسة اقترحت الإمكانية لإستخدام تحليل الـ Probit analysis لتحديد الجرعة المناسبة من أشعة جاما، بالإضافة لاستخدام المعلمات الجزيئية RAPD و ISSR لتحديد الإختلافات الوراثية.