DETECTION OF GENETIC EFFECTS IN γ-IRRADIATED GARLIC (*Allium sativum* L.) USING CYTOGENETIC, BIOCHEMICAL AND RAPD ANALYSIS

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Garlic (*Allium sativum* L.), belongs to *Liliaceae* family, it is a common food spice, used widely in many parts of the world. For many centuries, various species of genus *Allium* have been used as vegetables, spices, and as folk medicines for the curing of various diseases (Akgu, 1993). Garlic has been a subject of considerable interest as a medicine worldwide since ancient times. Also, garlic has been used worldwide as a seasoning spice and herbal remedy (Ahmad, 1996). Garlic is known to possess a vast variety of biological functions. It was reported as an antimicrobial (Kim, 2002), antithrombotic (Block *et al*., 1986), anticancer (Mousa, 2001) and antioxidant (Wu *et al*., 2001). It could improve the immune-system (Kang *et al*., 2001) as well as it had a capacity to lower serum lipid and glucose levels (Lawson *et al*., 2001). Garlic has demonstrated beneficial effects in a large number of pathological conditions, including hyperlipidemia (Jabbari *et al*., 2005), cardiovascular disorders and arteriosclerosis (Rahman and Lowe, 2006). Cancer preventative properties of garlic had also been reported (Ejaz *et al*., 2003). Epidemiologichal studies have revealed the lower risk of stomach cancer in people with high garlic intake (Galeone *et al*., 2006).

The diploid number of common garlic is 16 (2n=16) with a karyotypic formula of six metacentric chromosomes, four submetacentric chromosomes and six acrocentric chromosomes (Bozzini, 1991). The karyotype of Egyptian garlic clones seems to be different when compared with other genotypes grown worldwide (El-Mamlouk *et al*., 2002; Ata, 2005; Osman and Moustafa, 2009). The mitotic Index (MI) and the mitotic parameters, reflect chromosomal aberrations in several organisms including garlic (Swanson *et al*., 1990; Kaushik, 1996).

Gamma rays (is a part of electromagnetic spectrum) belongs to ionizing radiation can be energetically charged particles, such as electrons, or high-energy photons. The biological effect of gamma rays is based on the interaction with atoms or molecules in the cell, particularly with water to produce free radicals in cells. These radicals can damage or modify important components of plant cells and have been reported to affect differentially the morphology, anatomy, biochemistry and physiology of plants (Kim *et al*., 2004; Wi *et al*., 2005). The primary effects of ionizing radiation are ionization, dissociation and excitation. The excitation cause a weak interaction and the ioniza-

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tion and dissociation resulted in strong interaction. Absorption of ionizing radiation in biological materials acts, there is a possibility that it will act directly on critical targets in the cell (Kovacs and Keresztes, 2002).

Genetic characterization of garlic clones maintained in germplasm collections has been accomplished using various molecular markers, such as isozyme, AFLPs, and randomly amplified polymorphic DNA (RAPD) markers. Isozymes are co-dominant markers, but the number of isozyme markers is quite limited in garlic and some of them are stage-specific (Pooler and Simon, 1993; Ipek *et al*., 2003). On the other hand, RAPD markers are abundant (Maass and Klaas, 1995; Bradley *et al*., 1996; Ipek *et al*., 2003) and RAPD is an easily applicable marker system for the genetic characterization of garlic, but this marker system's reproducibility has been criticized (Karp *et al*., 1996).

The advantages of RAPD assays lie in the PCR-based technique being easy identification of the regions of amplification, deletion or rearrangement without prior information about the genome; this technique can be performed on only small amounts of DNA (Theodorakis and Bickham, 2004; Wolf *et al*., 2004). The technique has already been applied successfully to estimate DNA changes and genomic template stability (GTS). Loss of normal bands and appearance of new bands compared with control plant may reveal that the survival of the individuals was greatly affected (Cenkci *et al*., 2009). No previous references explaining the relationship between γ-irradiation and garlic RAPD polymorphism were found.

The objectives of the current study are to investigate cytogenetic, biochemical and molecular alterations of two Egyptian garlic cultivars (*Allium sativum* L.) after exposure to different doses of γ-irradiation and to compare GTS values calculated from the changes in RAPD profiles.

MATERIAL AND METHODS

This experiment was carried out at the laboratory of Genetics Dept., Fac. of Agric., Kafrelsheikh Univ., Kafr El-Sheikh, Egypt during two seasons (2013 and 2014).

Plant material

Two garlic (*Allium sativum* L.) cultivars: Balady and Sids-40 were used throughout this study. The garlic cloves were obtained from the Agricultural Research Center, Sakha, Kafr El-Sheikh, Egypt.

Plant cultivation

Relatively big garlic bulbs, were randomly chosen from Balady and Sids-40 cultivars, were divided into eight groups, each contained 250 g of cloves. These groups were exposed to eight gamma rays doses; 500, 750, 1000, 1250, 1500, 2000, 4000 and 8000 rad and un-irradiated group was added to the experiment as a control group. These treatments were ap-

plied for two seasons at the Nuclear Res. Center, Inshas, Egypt during 2013 and 2014. Gamma rays were emitted from 137 CS source by the use of GC40 model and dose rate: 0.996 rad/sec., irradiation chamber was 40 cm diameter and 10 cm height. Bulbs were packaged in paper bags, covered with aluminum foil, and exposed to gamma doses. Cloves of garlic used in this investigation were germinated in plastic pots under natural light.

Mitotic index (MI) analysis

For mitotic studies, acetocarmin-squashed preparations were made from the cooling-pretreated root tips of nine cloves for each treatment in one factor complete randomized design. Microscopic examination was done for mitotic index for each set of treatments and control. Mitotic index was calculated by using the following formula:

$$\text{Mitotic Index (MI)} = \frac{\text{Total number of dividing cells}}{\text{Total number of counted cells}} x100$$

Cells with mitotic chromosomal irregularities (ex. C-metaphase, chromatin stickiness, ring chromosome, bridges, laggards and fragments, etc…) were scored at all phases. The good mitotic spreads were photographed using the SIS computer program with OLYMPUS camera 4040.

Statistical analysis

Data were represented as mean ± standard error (SE) of at least three independent replicates for each experiment. Analysis of variance (ANOVA) followed by Duncan's multiple range test (Duncan, 1955) was used to determine the significant differences among the data. Differences were considered significant when $P \leq 0.05$. All statistical analyses were carried out using the Microsoft Excel software 2010.

Change in protein banding pattern induced by gamma rays in garlic plants

Proteins were extracted from M_2 gamma rays treated plants from both cultivars as well as from the non-treated plants (control) in two seasons (2013 and 2014). SDS-polyacrylamide gel electrophoresis was performed for the total proteins of leaves according to the method described by laemmli (1970). Leaf samples (250 mg) were grounded in liquid nitrogen and 500 µl of ice cold extraction buffer (0.05M Tris-HCL buffer PH 6.8 containing 20% sucrose and 5mM dithiotritol) were added, and then centrifuged at 12,000 rpm for 10 min. at 4°C. Following electrophoresis, the gel of total protein was stained for 2 h. with Coomassie Brilliant Blue G-250. The molecular weight (MW) of the detected bands was determined using PiNK prestained protein ladder (175, 130, 90, 70, 60, 50, 40, 30, 20 and 15 KDa).

Genomic DNA extraction

Total genomic DNA was extracted from fresh young leaves of the M_2 treated plants and their original parents (Balady and Sids-40). After freezing by liquid ni-

trogen, leaves were grounded in a mortar with a pestle until no large pieces could be seen. One gram of the powder was used for DNA extraction by Cetyltrimethyl Ammonium Bromide (CTAB) according to Murray and Thompson (1980). DNA was air-dried, dissolved in TE buffer and stored at -20°C until polymerase chain reaction (PCR) amplification was performed. DNA quality was tested using 1.5% agarose gel electrophoresis. For quantifying the amount of DNA, 20 µl of stock nucleic acid were added to 480 µl of TE buffer and mixed, absorption (OD) was read in a spectrophotometer at wavelength of 260 and 280 nm.

RAPD-PCR procedures

Eight of 10-mers arbitrary RAPD primers were used in this study and the list of primers code and sequences is shown in Table (1).

PCR was carried out in presence of 1X Taq DNA polymerase buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$), 100 M dNTPs, 5 picomole single random primers, 30 ng template DNA, 0.5 unit of Taq DNA polymerase in a total volume of 25 µl. PCR amplification was performed in PTC-100 thermal controller (MJ- Resarch, INC) programmed as follow, 94°C for 3 min followed by 30 cycles for 30 sec for denaturation at 94°C, 30 sec for annealing at 30°C and 1 min for polymerization at 72°C, followed by a final extension step at 72°C for 5 min. The amplification products were resolved by electrophoresis in 1.5% agarose gels in 0.5 X TBE buffer and documented on Gel Documentation UVP, US/Canada.

Estimation of genomic template stability (GTS)

Each RAPD-PCR profiles (disappearance of bands, appearance of new bands and variation in band intensities in comparison with control profiles) was considered in order to assess any DNA damage and the template genomic stability (percent) was calculated for each treatment for each primer. Genomic template stability (GTS, %) was calculated as follows:

$$GTS\ (\%) = (1 - a/n) \times 100$$

Where "a" is the number of RAPD polymorphic bands detected in each treatment and "n" is the number of total bands in the control. Polymorphisms observed in the RAPD profile include disappearance of a normal band and appearance of a new band in comparison with control profile (Atienzar *et al*., 1999).

RESULTS AND DISCUSSION

Bulbs of the two cultivars (Balady and Sids-40) of *Allium sativum* that were exposed to different doses of γ- radiation were planted and irrigated for 15 days. Exposure of garlic bulbs to γ- radiation caused obvious changes in plant growth. The lowest dose of γ- radiation (500 rad) treatment and the untreated one only were able to germinate in the two seasons of the experiment (2013 and 2014). Variable cytogenetic, biochemical, and molecular parameters have been extensively studied.

Cytogenetic effect of γ-irradiation on mitosis of garlic root tips

1. Effect of γ-irradiation on mitotic index (MI)

Mitotic index was used to determine the rate of cell division in garlic root tips of M_1 seedlings. The mitotic index (MI) values of the two studied garlic genotypes are presented in Table (2). From the obtained results, it was surprising to notice that both the lowest and highest γ- radiation doses (500 and 8000 rad, respectively) caused increase in MI values in both used garlic cultivars (Balady and Sids-40) while, the dose of 4000 rad caused decrease of MI in both garlic cultivars. Meanwhile, the four γ-radiation doses 500, 1000, 1250 and 2000 rad caused significant increase and the three doses 750, 1500 and 4000 rad caused significant decrease in MI in Sids-40 cultivar. For Balady cultivar, three γ-radiation doses (500, 1500 and 8000 rad) caused significant increase in MI, but the two doses (1250 and 2000 rad) caused significant decrease in MI. For Balady cultivar, mitotic index reached to its maximum values (10.68 ± 1.63 and 10.09 ± 2.71) which did not differ significantly, at 500 and 8000 rad doses, respectively and (13.07 ± 2.04) for Sids-40 cultivar at 1000 rad. This means that γ-irradiation doses prolonged cell division time and shortened interphase in cell division, so that division cycle was shortened. The reduction in mitotic index clearly indicate the prolongation of cell generation time. The means of mitotic index (MI) were significantly different among the applied γ-irradiation doses. Some doses exhibited high variability in the percentage values of mitotic stages. This might be due to differences in genetic control systems of mitosis (cell cycle program) and/or the quantity of somatic mutations (Kaushik, 1996; Yasuhara and Shibaoka, 2000).

2. Effect of γ-irradiation on induction of chromosomal aberrations

Another form of cytogenesis instability associated with irradiated garlic was chromosomal structure changes. Aberrant chromosomal structures such as c-metaphase, fragmentation, disrupted metaphase, disrupted anaphase, formation of bridges, laggard, polyploidy, lagging chromosomes, ring chromosomes, binuclei cells, multinuclei cells, non-orientated cells, multipolar anaphase and stickiness as well as large cells were detected in most γ-irradiation doses.

Chromosomal aberrations were scored in different mitotic stages of *Allium sativum* root tips of M_1 seedlings and the cytological effect was estimated as the percentage of cells showing chromosomal abnormalities (Table 2). For Balady cultivar, summarized results in Table (2) show that the frequency of abnormalities was significantly increased as the doses of γ-irradiation increased from 500 to 1250 rad, while the doses over 1250 rad decreased the abnormality percentage, expect in the case of 8000 rad the abnormality percentage was increased again to 50.30 ± 5.0. While, no correlation between the abnormality percentage and the doses of γ-irradiation was found in the case of gar-

lic cultivar Sids-40 (Table 2).The highest values of abnormalities (71.65 ± 7.01 and 69.14 ± 7.82%) were recorded for the doses of 1250 and 4000 rad, for Balady and Sids-40 cultivars, respectively.

It was of specific interest to notice that the γ-irradiation dose of 4000 rad which caused the significant decrease in MI in garlic cultivar Sids-40 gave also the highest values of chromosomal abnormalities in the same cultivar.

As presented in Tables (3 and 4) and Fig. (1), garlic root tips revealed various types of chromosomal aberrations as a result of treatment with γ-irradiation doses. The most frequent aberrations were c-metaphase, fragments, disturbed anaphase, laggards, disturbed metaphase, lagging and formation of bridges. These abnormalities may be due to genetic recovery, elimination of the abnormal cells or to genetic or chromosomal alternation. These results agree with those of Chan (1966) on Rosa spp, Mohamed (1980) on garlic, Gohil and Kaul (1983) and Talavera *et al*. (2003) on onion and garlic. They indicated that gamma rays induced different kinds of chromosomal aberrations.

Types of aberration such as chromosome bridges were found in the anaphase or telophase cells. Some of these were single bridge, while others were two or more bridges. A mitotic bridge is considered as a result of dicentric chromosomes or chromosomal stickeness (Elghamery *et al*., 2000). Bridges might have arisen through breaks in two chromosomes followed by union of the centric fragments (Shreekrishna, 2006) or due to stickiness of chromosomes at metaphase and their failure to separate at anaphase or due to breakage and reunion of chromosome (Badr, 1988; Grant, 1978).

Lagging chromosomes may be explained on the basis of abnormal spindle formation and failure of chromosome movement (Haiba *et al*., 2011). Types of abnormalities such as stickeness, disturbed anaphase, C-metaphase, bridges, laggards as observed in the present investigation point out to direct effect of γ-irradiation on spindle apparatus causing defective formation of the spindle fibers (Polit *et al*., 2000; Usciati *et al*., 2004). Fragments might have arisen due to the stickiness of the chromosomes and the consequent failure of the arrival of chromatids at the poles. Fragments may also be acentric chromosomes formed as a result of inversion (Agarawal and Ansari, 2001).

Micronuclei were in fact, the result of lagging and unoriented types of aberrations. They are small cytoplasmic substances, which are formed as a result of chromosome breaks and aneuploidy during cell division (Grisolia and Starling, 2001).

Binuclear cells were also observed which were considered as indicative of the ability of γ-irradiation to interfere with spindle and cell wall formation. The effect of γ-irradiation in the present study is probably due to direct effect on viscosity of protoplasm and DNA proteins of the chromosomes as stated by Abdelrhman (1997).

The existence of chromosomal aberrations in genus *Allium* was considered to be an important source of garlic diversity (Al-Zahim *et al*., 1999; Vosa, 2000). The present results further showed a high relation between mitotic index and the percentage of cells with chromosomal abnormalities in the tested garlic materials.

All ionizing radiations inhibit mitosis. All mitosis are not stopped, cells in mitosis as far long as mid or late prophase when radiation is given, may complete their divisions. The inhibitory effect is dependent upon dose. Radiation also brings non-disjunction of centromers at metaphase and the unequal distribution of chromosomes to daughter cells at anaphase. In addition ionizing radiation causes lagging of chromosomes on the spindle at anaphase, an effect ascribed to changes brought about in the centromeres. Ionizing radiation promotes cross-over in the region of the centromere due to an effect of radiation on the centromere (Florencio *et al*., 2004).

Biochemical effects of γ-irradiation on leaf protein of garlic

The biochemical and molecular studies were applied only on the 500 rad γ-irradiation treatment and untreated plants, which were able to germinate in the two seasons (2013 and 2014) as mentioned before. SDS-PAGE analysis was carried out on M2 leaf seedling protein of garlic plants whose parents were previously treated with 500 rad of γ-irradiation. The protein banding pattern was illustrated in Fig. (2) and Table (5). The obtained data revealed that changes in protein banding patterns were very high. Comparing with the untreated plants (control), the recorded changes were expressed as variations in the number of separated bands, disappearance or appearance of certain bands and alterations in bands intensity.

The total number of bands was 18 bands and the molecular weight of protein bands ranged from 8 to 174 KDa. In the first season (2013), protein profile of the control was found to have 14 and 16 bands for Balady and Sids-40, respectively, out of them 12 bands were found to be common in all treatments (monomorphic bands) for this season. On the same hand, results of the second season (2014) showed decrease in the number of bands for total protein, where the control exhibited seven bands for both cultivars and three of them were found to be monomorphic bands.

A specific band with MW of (8 KDa) was appeared; this band was polymorphic, since it did not appear in control plants but appeared in the 500 rad treatment for the two cultivars in the first season (2013). On the other hand, some bands were appeared in control but disappeared in the treated plants. The disappearance of these bands could be explained on the basis of mutational event at the regulatory genes that prevent or attenuate transcription (Muller and Gottschalk, 1973). Induction of laggards, bridges and micronuclei by γ-irradiation may lead to

the loss of genetic material. Therefore, some electrophoretic bands were disappeared due to the loss of their corresponding genes (Abdelsalam *et al*., 1993). However, the highest number of protein bands (18 bands) was recorded in garlic plants treated with 500 rad.

Considering bands intensity, remarkable variations between the control and the treatments were observed. There was a decrease in the intensity of the main monomorphic band with MW of 49 KDa in protein profile produced by the control and all treatments. Intensity of the smallest band with MW of 8 KDa was increased as a result of treatment with 500 rad in Balady cultivar at the first season. The increase in bands intensity could be attributed to gene(s) duplication that resulted from cytological abnormalities induced by application of γ-irradiation. The presence of laggards and bridges support this conclusion. This conclusion is in agreement with those of Gamal El-Din *et al*. (1988) who noticed that increasing the number of genes encoding for the different protein subunits through doubling of chromosome number from 12 to 24 in *Vicia faba* caused an increase in band intensity. Based on previous research reports, the total protein and carbohydrate contents were decreased with increasingly higher dosage of γ-irradiation caused by higher metabolic activities and hydrolyzing enzyme activity in germinating seed (Barros *et al*., 2002; Maity *et al*., 2004). Total proteins and carbohydrates were decreased with increasing high γ-irradiation dosage in wheat and rice plants (Hagberg and Persson, 1968; Inoue *et al*., 1975).

Molecular effects of gamma rays on polymorphism based on RAPD markers

In order to identify the genetic variations between the two genotypes under control and treatment with 500 rad in two seasons, eight random primers were used. All the eight primers gave reproducible PCR products with a clear pattern for each treatment and showed informative and easily scrabble RAPD profiles (Fig. 3). These primers produced multiple band profiles with a number of amplified DNA fragments varying from 7 to 11 as shown in Table (6). The highest number of bands (11) was generated by using the primer OPB-06, while the lowest number was seven bands and generated with primers OPA-06 and OPA-07. The total number of generated bands was 69 bands, 50 out of them were polymorphic as shown in Table (6).

The amplification products obtained by RAPD-PCR showed various bands with different lengths. The used RAPD primers in our study yielded specific and stable results and they indicated that the used primers have a discrete efficiency to amplify the genomic DNA of garlic (Fig. 3).

The disappearance or the loss of PCR amplification products can reveal changes in the DNA sequence due to mutations, showing new annealing events and/or large deletions, bringing two pre-existing sites nearer or separating them

farther. Furthermore, the amplification pattern of the DNA showed the acquisition or the loss of bands and/or the change of intensity of the same, caused by a variation in the number of recognition sites of the sequence of the primer and thus of mutations. Variations of the band frequency could be the result of structural changes induced by the gamma rays.

Yoko *et al*. (1996) studied the effect of γ-irradiation on the genomic DNA of corn, soybean, and wheat. They concluded that large DNA strands were broken into small strands at low irradiation doses but small and large DNA strands were broken at higher irradiation doses. This observation was also stated by Artık and Pekşen (2006) who found a reduction in *Vicia faba* seed yield and harvest index in some varieties when seeds were treated with relatively low doses 25 and 50 Gy of γ-irradiation.

Genomic template stability (%) Evaluation

Changes in the RAPD patterns are expressed as decreases in GTS%, a qualitative measure reflecting the change in the number of RAPD profiles generated by the γ-irradiation treated plants, in relation to profiles obtained from the control. GTS values are reported in Table (7). According to our results, the RAPD profiles of treated and untreated groups showed differences in banding patterns. When the control and treatments are compared, these differences observed in all RAPD profiles are clearly exhibited by the appearance/disappearance of some bands. The maximum change in RAPD profiles (disappearance b and/or appearance a) was obtained in Balady in season 2014 when compared with untreated plants. Also, it observed that the higher percentage of GTS was observed in sids-40 in season 2014, while the lowest percentage was recorded for Balady cultivar in season 2014. The appearance/disappearance of normal bands may be related to the events such as DNA damage, point mutations and/or complex chromosomal rearrangements induced by gamma rays (Wolf *et al*., 2004; Atienzar *et al*., 2002).

Disappearance of bands are likely to be due to changes in oligonucleotide priming sites, originating from rearrangements and less likely from point mutations and DNA damage in the primer binding sites (Liu *et al*., 2005; Enan, 2006; Liu *et al.*, 2009). The disappearance of a normal RAPD product may be related to the events such as DNA damage (example, single and double strand breaks, modified bases, a basic sites, oxidized bases, bulky adducts, DNA–protein cross-links), point mutations and/or complex chromosomal rearrangements induced by genotoxins (Atienzar *et al*., 1999).

SUMMARY

The effect of different doses of gamma irradiation on cytogenetic and biochemical characters was studied in two Egyptian cultivars of garlic (Balady and Sids-40). Identification of DNA polymorphism among the treatments through a Randomly Amplified Polymorphic DNA (RAPD) marker analysis was also of in-

terest in this study. The cytological analyses showed that the highest percentage value of mitotic index (MI) was recorded for cultivar Sids-40 (13.07 ± 2.04) at 1000 rad, while the dose 1250 rad exhibited the lowest value (4.65 ± 0.43) for Balady cultivar. It was of specific interest to notice that the γ-irradiation dose of 4000 rad which caused the significant decrease in MI in Sids-40 garlic cultivar gave also the highest values of chromosomal abnormalities in the same cultivar. Both the lowest and highest γ-irradiation doses used (500 and 8000 rad, respectively) caused increase in MI in both garlic cultivars. While, the 4000 rad dose caused decrease in both garlic varieties. On the other hand, all doses of gamma rays induced significant increase in the percentage of chromosomal abnormalities which examined at different mitotic stages and the most frequent aberrations were c-metaphase and fragments. Data showed that cultivar Balady had the highest values of total chromosomal abnormalities at 1250 rad, while Sids-40 showed the highest value at 4000 rad. The biochemical studies exhibited changes in protein banding patterns; these changes included alterations in number of bands, band intensity and disappearance or appearance of certain bands. The occurred changes in RAPD profiles using eight primers following γ-irradiation treatment included variation in band intensity, loss of normal bands and appearance of new bands compared with the untreated plants. These results indicated that polymorphism and genomic template stability (GTS) value was affected at the above gamma doses. In conclusion, DNA polymorphisms detected by RAPD analysis could be used as a useful biomarker assay for the detection of genotoxic effects of γ-irradiation on plants.

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Table (1): Codes and sequences of the RAPD primers used for detection of genetic variation of induced garlic mutants and their parents.

No.	Primer code	Primer sequences 5′→ 3′
1	OPA-06	GGTCCCTGAC
2	OPA-07	GAAACGGGTG
3	OPA-09	GGGTAACGCC
4	OPA-14	TCTGTGCTGG
5	OPB-01	GTTTCGCTCC
6	OPB-06	TGCTCTGCCC
7	OPB-08	GTCCACACGG
8	OPB-17	AGGGAACGAG

Table (2): Effect of γ-irradiation on mitotic cell division and chromosomal abnormality percentages in root tips of Balady and Sids-40 garlic varieties.

Doses of gamma rays	Balady			Sids-40		
	Total number of examined cells	Mitotic index %	Total abnormality %	Total number of examined cells	Mitotic index %	Total abnormality %
Control	5049	7.69 ± 1.08 abc	9.88 ± 2.37 e	1863	8.69 ± 0.67 bcd	9.97 ± 2.98 f
500 rad	4177	10.68 ± 1.63 a	24.09 ± 3.25 d	7023	10.10 ± 0.73 ab	30.04 ± 3.26 de
750 rad	4173	8.22 ± 0.98 abc	38.97 ± 5.20 bc	5787	6.50 ± 0.88 de	43.74 ± 4.43 cd
1000 rad	2180	8.81 ± 2.51 abc	40.12 ± 7.35 bc	1672	13.07 ± 2.04 a	29.66 ± 3.30 e
1250 rad	2075	4.65 ± 0.43 c	71.65 ± 7.01 a	9211	9.68 ± 1.11 bc	39.35 ± 4.59 de
1500 rad	4728	9.35 ± 0.84 ab	35.16 ± 4.65 cd	6095	6.82 ± 1.18 cde	68.00 ± 3.69 ab
2000 rad	2788	5.38 ± 0.48 bc	33.51 ± 4.18 cd	3663	9.87 ± 1.00 bc	37.13 ± 3.71 de
4000 rad	3903	6.80 ± 1.29 abc	31.67 ± 3.65 cd	2935	4.84 ± 0.57 e	69.14 ± 7.82 a
8000 rad	2403	10.09 ± 2.71 a	50.30 ± 5.00 b	5235	8.89 ± 0.81 bcd	54.45 ± 7.91 bc

Values followed by the same letter are not significantly differ at 0.05 probability level. No. : normal Ab.: abnormal

Table (3): Frequencies of abnormal chromosome behavior in root tip cells of garlic (Balady cultivar) after treatment with different doses of γ-irradiation.

Doses of gamma rays	Total no. of aberrant cells	Bridges %	Polyploidy %	Binuclear %	Multinuclear %	c-metaphase %	Fragments %	Ring ch. %	Lagging ch. %	Large cells %	Non-oriented cells %	Stickiness %	Laggard ch. %	Disturbed anaphase %	Disturbed metaphase %	Multibolar anaphase %
500 rad	102	4.9	-	4.9	0.98	29.4	12.7	-	9.8	1.9	0.88	-	9.8	8.8	13.7	-
750 rad	132	4.5	-	16.0	-	27.2	12.1	0.75	11.7	3.0	3.00	-	7.5	9.0	9.0	6.8
1000 rad	83	7.2	-	7.2	-	18.0	12.0	1.20	7.2	2.4	-	1.20	12.0	10.8	13.2	7.2
1250 rad	91	7.6	1.1	6.5	-	21.9	10.9	1.10	8.7	3.2	2.10	4.30	12.0	7.6	9.7	4.3
1500 rad	182	6.5	1.6	3.8	1.09	9.8	20.3	-	3.2	2.1	1.60	2.70	14.2	10.9	11.5	9.8
2000 rad	79	8.8	-	3.8	1.20	25.3	13.9	1.20	6.3	1.2	-	1.20	11.3	24.0	12.6	8.8
4000 rad	66	15.1	-	4.5	-	15.1	16.1	-	10.6	1.5	3	1.50	13.1	15.1	-	4.5
8000 rad	83	8.4	-	7.2	3.60	9.6	19.2	-	12.0	1.2	-	-	12.0	12.0	10.8	4.8
Total	818	60.0	4.0	44.0	7.00	148.0	124.0	4.0	64.0	18.0	12.00	12.00	93.0	96.0	87.0	51.0
		7.3	0.48	5.3	0.85	18.09	15.15	0.48	7.82	2.20	1.47	1.47	11.37	11.74	10.64	6.23

Table (4): Frequencies of abnormal chromosome behaviors in root tip cells of garlic (Sids-40 cultivar) after treatment with different doses of γ-irradiation.

Doses of gamma rays	Total no. of aberrant cells	Bridges %	Polyploidy %	Binuclear %	Multinuclear %	c-metaphase %	Fragments %	Ring ch. %	Lagging ch. %	Large cells %	Non-oriented cell %	Stickiness %	Laggard ch. %	Disruptd anaphase %	Disruptd met-aphase %	Multipolr an-aphase %
500 rad	198	8.0	3.00	4.50	1.5	25.7	23.2	-	11.1	-	2.50	2.00	2.50	4.00	4.5	7.10
750 rad	139	5.8	0.70	5.00	0.7	23.1	21.5	-	17.9	1.40	0.70	0.70	10.00	5.00	4.3	2.80
1000 rad	67	11.1	1.40	1.40	-	7.4	38.8	-	10.4	-	1.40	-	7.40	5.90	13.4	5.90
1250 rad	293	10.2	0.68	0.68	2.0	22.8	41.9	0.68	11.9	1.30	0.34	1	4.09	4.09	4.7	3.10
1500 rad	242	3.3	0.82	3.30	-	21.4	35.1	0.82	9.0	-	-	0.82	4.90	4.50	4.1	4.10
2000 rad	113	7.0	-	3.50	-	12.3	23.8	0.88	14.1	-	1.70	-	5.30	12.30	8.8	6.10
4000 rad	187	3.7	1.60	5.30	1.6	12.2	13.3	1.16	3.7	1.60	2.10	-	3.70	4.80	4.8	1.06
8000 rad	309	5.8	2.50	1.60	-	16.8	38.1	0.64	10.6	0.32	5.10	2.50	3.20	2.90	3.5	2.2
Total	1548	99	23	46	13	296	482	9	31	18	31.0	18	71	74	78	57
		6.39	1.49	2.97	0.84	19.12	31.14	0.58	2.00	1.16	2.00	1.16	4.59	4.78	5.04	3.68

Table (5): Electrophoresis of total protein banding patterns of garlic leaf protein after treatment by 500 rad γ-irradiation dose.

No. of bands	No. of Samples							
	Balady 2013		Sids-40 2013		Balady 2014		Sids-40 2014	
	Control	500 rad	Control	500 rad	Control	500 rad	Control	500 rad
1	+	+	+	+	-	-	-	-
2	-	+	+	-	-	-	-	-
3	-	+	+	-	-	-	-	-
4	+	+	+	+	-	-	-	-
5	+	+	+	+	-	-	-	-
6	-	+	+	-	-	-	-	-
7	++++++++++	++++	+++++++++	++++++	+++++	+++++	++++++	+++++
8	++++	++++	++++	++++	+	+	++	-
9	+++	++	+++	+++	++	++	+++	-
10	+++	++	++	++	++	-	++	-
11	++	++	++	++	-	-	-	-
12	+	+	+	+	-	-	-	-
13	+	+	+	-	-	-	-	-
14	+	+	-	-	-	-	-	-
15	+	+	+	+	-	-	-	-
16	+++	++	++	++	+	+	++	++
17	++	++	++	++	++	++	++	+
18	-	+++++++	-	+	+	+	+	-
Total	14	18	16	13	7	6	7	4

-, absent; +, very weak; ++, weak; +++, intermediate; ++++, strong; +++++, very strong; up to +++++, very high strong.

Table (6): Distribution of RAPD markers among the treated and untreated plants.

Primer code	Total number of bands	Polymorphic bands	Polymorphism %
OPA-06	7	6	85.71
OPA-07	7	7	100.00
OPA-09	9	7	77.78
OPA-14	9	7	77.78
OPB-01	9	6	66.67
OPB-06	11	8	72.73
OPB-08	8	6	75.00
OPB-17	9	3	33.33
Total	69	50	72.46

Table (7): Genomic DNA template stability % as revealed from RAPD profiles after treatment with 500 rad γ-irradiation comparing with control in two garlic genotypes over two seasons.

Primer code	Balady									Sids-40								
	Total number of control bands	500 rad								Total number of control bands	500 rad							
		Season 2013				Season 2014					Season 2013				Season 2014			
		a	b	c	d	a	b	c	d		a	b	c	d	a	b	c	d
OPA-06	3	5	2	0	0	4	1	0	0	4	4	1	0	1	3	2	1	1
OPA-07	6	1	2	1	1	1	2	1	0	6	3	2	0	1	1	6	0	0
OPA-09	7	2	1	0	1	3	2	0	1	4	7	2	0	2	5	2	0	2
OPA-14	6	4	2	1	2	2	2	0	3	5	5	1	1	1	0	1	1	0
OPB-01	7	3	1	4	1	4	1	5	0	9	0	5	0	3	2	3	1	5
OPB-06	11	0	4	2	4	0	6	1	3	4	0	0	0	0	3	0	0	2
OPB-08	6	2	4	1	1	4	0	2	2	8	1	1	1	1	0	0	0	0
OPB-17	7	1	0	4	1	4	2	4	0	9	0	2	2	1	0	2	2	1
Total	53	18	16	13	11	22	16	13	9	49	20	14	4	10	14	15	5	11
a+b+c+d		58				60					48				45			
a+b		34				38					34				29			
GTS(%)		35.8				28.3					30.6				40.8			

a: appearance of new band, b: disappearance of normal band, c: increase in band intensity, d:decrease in band intensity,
a+b: polymorphic bands, GTS: genomic template stability.

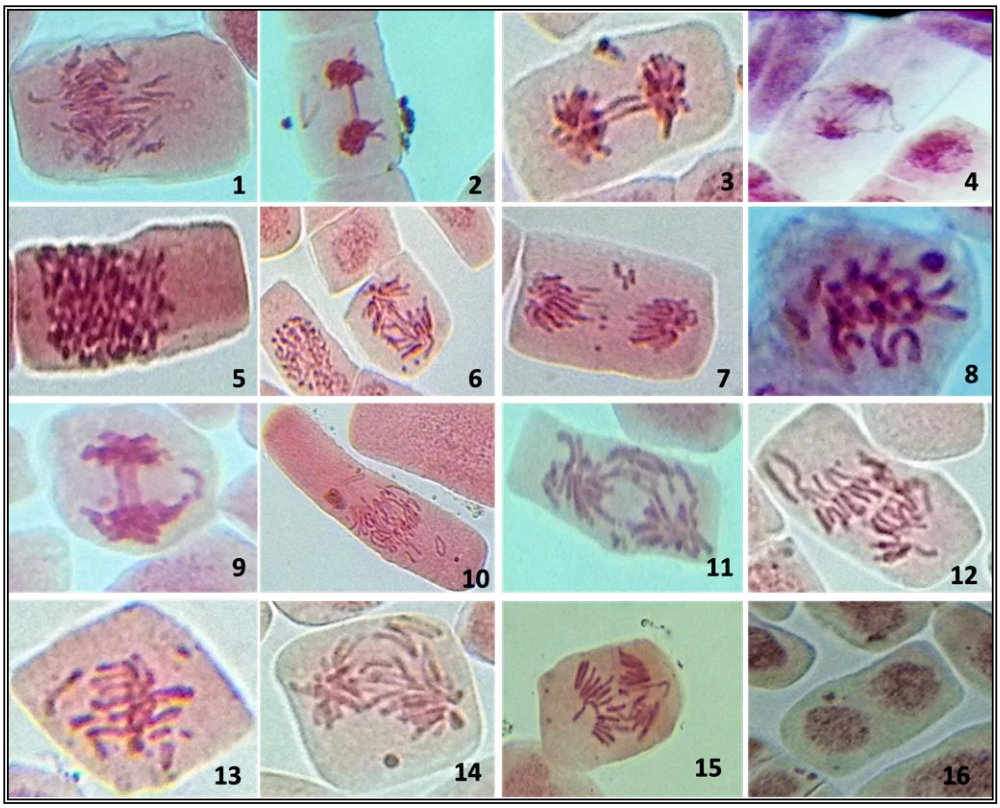

Fig. (1): Types of chromosomal aberrations observed in M1 root tips of garlic cultivars treated with different doses of γ-irradiation (1) Ring chromosome with laggard chromosome, (2) Single bridge with sticky chromosomes, (3) Double bridge, (4) Multible bridge with ring chromosome, (5) Polyploidy, (6) Binuclear cell with multible bridge and disrupted anaphase, (7) Multinuclear cell with fragments, (8) C-metaphase, (9) Stickiness chromosome and chromosome breakage, (10) Large cell with ring chromosome, (11) Non-oriented cell with bridge, (12) Disrupted anaphase with laggards, (13) Disrupted metaphase with fragments and laggards, (14) Nuclear cell, (15) Multipolar anaphase with lagging chromosome and (16) Micronucleus with binucleate cell.

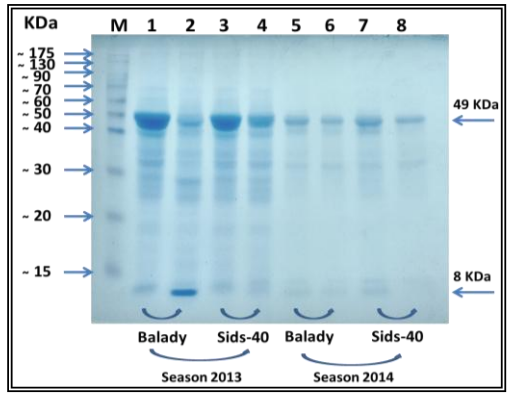

Fig. (2): Protein banding pattern showing untreated and treated garlic plants with 500 rad. Lanes 1, 3, 5 and 7 are untreated plants and lanes 2, 4, 6 and 8 are 500 rad treated plants.

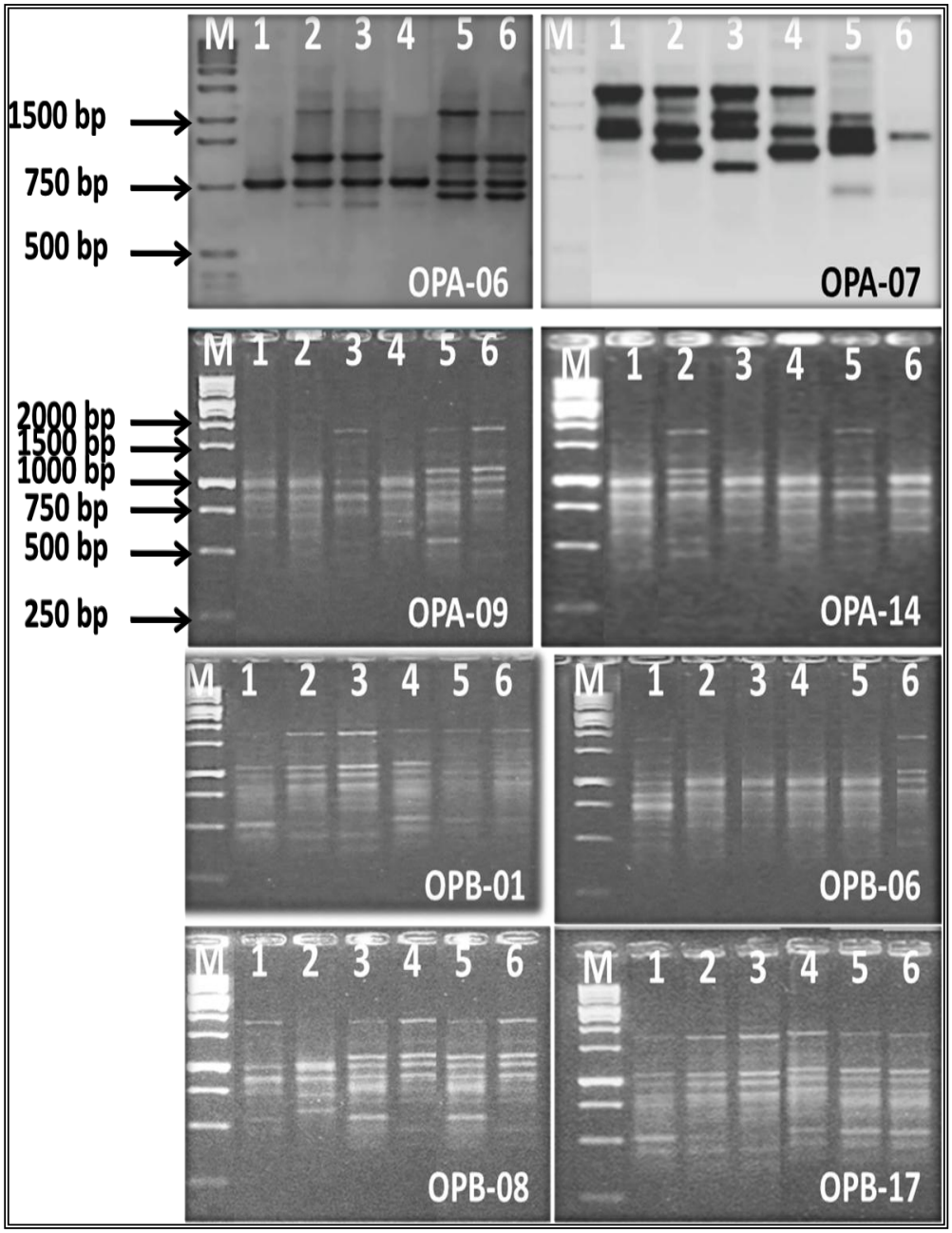

Fig. (3): RAPD profile demonstrating polymorphism between the two tested genotypes. M (1 kb DNA ladder), 1, 2, 3 Balady cultivar (1 control, 2 and 3: 500 rad treated garlic plants in seasons 2013 and 2014, respectively), 4, 5, 6 Sids-40 cultivar (4: control, 5 and 6: 500 rad treated garlic plants in seasons 2013 and 2014, respectively).