Effect of Gamma Irradiation on Enhancement of Some Economic Traits and Molecular Changes in *Hibiscus Sabdariffa* L.

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Abstract: Seeds of *Hibiscus sabdariffa* were irradiated with gamma rays (100, 200, 300, 400, 500, 600, 700 and 800 Gy) for determining the effectiveness of different doses of irradiation on growth behaviour, yield and evaluate of roselle calyx extract and quality. Gamma irradiation at 600 Gy was superior in growth criteria enhancement. Maximum mean values for fresh and dry weight of leaves, stems and roots/plant were recorded at 600 Gy in the first season and 500 or 600 in the second one. The application of 600 Gy gave the highest effect on increasing number of fruit per plant and the most significantly effective treatment for increasing anthocyanin. The significantly higher calyx yield per plant recorded by the application of 700 Gy. The variation in DNA of the irradiated seeds in comparison to the control were successfully assessed using random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR).
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1. Introduction

Hibiscus sabdariffa L., popularly known as roselle, is a member of the family Malvaceae and one of the most important and popular medicinal and industrial plants, the calyx is widely used for producing drinks or tea because of its high content of anthocyanins and organic acids (Hong and Wrostlad, 1990; Gomez-Leyva *et al*., 2008; Cissé *et al*., 2009) as well as flavour and colour additives in the manufacture of jam, liquor, and jellies (Akindahunsi and Olaleye, 2003). In ethno medicine, *H. sabdariffa* is traditionally used to deal with several health problems, including hypertension, pyrexia and liver disorders, microorganism growth; it is also used as a diuretic, sedative, or digestive (Faraji and Tarkhani, 1999; Chen *et al*., 2003; Akindahunsi and Olaleye, 2003). The positive physiological effect of this plant extract could be related to the presence of anthocyanins with potent antioxidant activity. Anthocyanins in addition to their colorful characteristics possess antioxidant properties (Francis, 2000).

The study of the effects of radiation on plants is a broad and complex field. Gamma irradiation was found to increase plant growth and development by inducing cytological, genetical, biochemical, physiological and morphogenetic changes in cells and tissues depending on the irradiation level (Gunckel and Sparrow, 1961). It is one of the important physical agents used to improve the characters and productivity of many plants (Jaywardena and Peiris, 1988, Sharma and Rana, 2007). The gamma ray had adverse effect on traits of plants and this depended on plant species or varieties and the dose of irradiation (Artk and Peksen 2006). These effects include changes in the plant cellular structure and metabolism e.g., dilation of thylakoid membranes, alteration in photosynthesis, modulation of the antioxidative system and accumulation of phenolic compounds (Kim *et al*., 2004, Wi *et al*., 2005). Mokobia and Anomohanran 2005) found that gamma irradiation were very useful not only for sterilization of medicine but also for the preservation of food and cereals in nutrition and agriculture.

Irradiation also been successfully used for mutation in breeding of various crops and ornamental plants (Song and Kang, 2003) and has proven an adept means of encouraging the expression of recessive genes and producing new genetic variations (Schum, 2003; Song and Kang, 2003; Yoon *et al*., 1990). Many of the complications of a phenotypic or biochemical based assay can be mitigated through direct identification of genotypes with DNA based assays (Mengoni *et al*., 2000) One such method is RAPD-PCR (random amplified polymorphic DNA-polymerase chain reaction) which amplifies random

genomic DNA sequences using single, short arbitrary primers, and these can be effectively used as genetic markers. The RAPD technique therefore surveys (scans) numerous loci in the genome, which makes this method particularly attractive for analysis of genetic distance and similarity between closely related species (Persson and Gustavsson 2001 and Crockett *et al*., 2002).

The present work aimed to investigate the effect of different doses of gamma irradiation (0.0, 100, 200, 300, 400, 500, 600, 700 and 800 Gys) on growth, yield, calyx extract and quality as will as molecular changes of roselle plants.

2. Materials and methods

Roselle (*Hibiscus sabdariffa* L.) variety "Sabahia 17" used in this investigation. Seeds of roselle were obtained from Medicinal and Aromatic plants Departmet, Horticulture Research Institute, Agriculture Research Center, Giza, Egypt. Dry seeds were divided into nine groups. The first group was kept without irradiation as control, while the rest were exposed to 100, 200, 300, 400, 500, 600, 700 and 800 Gray gamma-irradiation doses using Egypt's Mega Gamma-1 type J 6600 cobalt-60 irradiation at Cyclotron Department, Nuclear Research Center, Atomic Energy Authority, Egypt.

A field experiment was conducted during the two successive seasons of 2009 and 2010 at the Faculty of Agriculture Experimental Station, Suez Canal University, Ismailia, Egypt. Seeds were sown in sandy soil on the 4th May for both seasons. Each treatment was planted in 6 rows, 4 m long and 0.6 m wide, making an area of 14.4 m^2. Hills were 50 cm apart; 5 seeds per hill then thinned, three weeks later to one plant/ hill. Other agricultural practices such as: irrigation and weeding were carried out as recommended.

Measurements were taken on

Vegetative growth characters

After 132 days from sowing before harvesting, the following growth criteria were recorded, using eight random plants from each treatment, plant height (cm), number of branches/ plant, main root length/ plant, number of roots/ plant, fresh and dry weight of leaves, stems and roots/ plant (g).

Yield components

In both seasons at harvest (180 days seed sowing), number of fruits/ plant and fresh and dry weight of calyx/ plant (g) were taken.

Chemical analysis in leaves and calyxes

Photosynthetic pigments:

The fourth leaf from top was picked after 132 days from sowing. Chlorophylls a and b in leaves were determined calorimetrically according to A.O.A.C. (1980).

Total anthocyanin in dried air harvested roselle calyx determined according to the method described by Fuleki and Francis (1968) and developed by Du and Francis (1973).

Genomic DNA extraction

A modified CTAB (hexadecyl trimethyl ammonium bromide) procedure based on the protocol of Porebski *et al.* (1997) was adopted for obtaining good quality total DNA. 100 mg of 40-days-old young fresh leaves of roselle was collected and quickly frozen in liquid nitrogen then ground using mortar and pestle. Five ml of CTAB extraction buffer (60ºC), 50 mg PVP (polyvinyl pyrolidone) and 15 µl β-Mercaptoethanol (0.3%) were added. The tubes were mixed by inversion and incubated at 65ºC for one hour. Then, 6 ml of chloroform: isoamyl alcohol (24:1) was added and contents were mixed by inversion to form an emulsion. The tubes were centrifuged at 5000 rpm for 20 min at room temperature. The top aqueous layer was further centrifuged at 5000 rpm after addition of 6 ml of chloroform: isoamyl (24:1). Half-volume of 5 M NaCl and two volumes of absolute cold ethanol were added to the supernatant and were mixed well. The tubes were incubated at –20ºC overnight, and then centrifuged at 8000 rpm for 15 min. The supernatant was discarded, the pellet was washed with 70% cold ethanol, and dried in Speed Vac (Savant, USA) for 10 min. The pellet was dissolved in 300 µl TE buffer (pH 8.0) overnight at 4º C. Then, it was transferred to 1.5 ml centrifuge tube. To remove RNA contamination, 4 µl (10 mg/ml) RNase (Sigma, USA) were added to the DNA solution and the mixture was incubated at 37ºC for 2 hours. The extracted DNA was deproteinized by adding 4 µl (1mg/ml) proteinase K (Sigma, USA) and was incubatied at 37ºC for 2 hours. Three hundred µl of Tris-saturated phenol-chloroform were added and mixed well by inversion. Tubes were centrifuged at 14000 rpm for 15 min in a centrifuge (Eppendorf, USA). The upper layer was transferred to new tube and 150 µl of TE buffer was added to the phenol phase, mixed, spun for 10 min, then the upper layer containing the DNA was removed and was added to the sample. DNA was precipitated overnight at –20ºC using 0.1 volume 3 M sodium acetate (pH 8.0) and two volumes of chilled absolute ethanol. Samples were centrifuged at 14000 rpm at 4ºC for 15 min. The DNA was washed with 70 % ethanol, briefly air-dried and re-dissolved in TE buffer.

Estimation of DNA Concentration:

DNA concentration was determined using NanoDrop 3300 (Thermo Scientific, Wilmington, USA).

Randomly Amplified Polymorphic DNA (RAPD)

RAPD-PCR Reactions

A set of twenty-eight random 10-mer primers (Table1) was used in the detection of polymorphism among the irradiated seeds and control. These primers were synthesized on an ABI 392 DNA/RNA synthesizer (Applied Biosystems) RAPD-PCR was carried out according to the procedure given by Williams *et al.* (1990) with minor modifications. The amplification reaction was carried out in 25 µl reaction volume containing 1X PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 1 µM primer, 1 U Go *Taq* DNA polymerase (Promega, USA) and 25 ng templates DNA.

Table (1): Sequence of the twenty-eight decamer arbitrary primers assayed in RAPD-PCR.

Name	Sequence (5´-3´)	Name	Sequence (5´-3´)
OPA-05	ACGGGTCTTG	OPD-02	GGACCCAACC
OPA-07	GAAACGGGTG	OPD-07	TTGGCACGGG
OPA-14	TCTGTGCTGG	OPD-07	TTGGCACGGG
OPA-16	AGCCAGCGAA	OPG-10	AGGGCCGTCT
OPB-02	TGATCCCTGG	OPH-05	AGTCGTCCCC
OPB-06	TGCTCTGCCC	OPO-15	TGGCGTCCTT
OPB-08	GTCCACACGG	OPO-20	ACACACGCTG
OPB-12	CCTTGACGCA	OPZ-12	TCAACGGGAC
OPB-13	TTCCCCCGCT	OPZ-13	GACTAAGCCC
OPk-01	CATTCGAGCC	OPZ-14	TCGGAGGTTC
OPk-02	GTCTCCGCAA	OPZ-15	CAGGGCTTTC
OPk-03	CCAGCTTAGG	OPZ-18	AGGGTCTGTG
OPk-12	TGGCCCTCAC	OPZ-19	GTGCGAGCAA
OPk-16	GAGCGTCGAA	OPZ-20	ACTTTGGCGG

Thermocycling profile and detection of the PCR products

PCR amplification was performed in a C1000- Thermocycler (*BIO RAD, USA*) programmed to fulfill 40 cycles after an initial denaturation cycle for 2 min at 94ºC. Each cycle consisted of a denaturation step at 94ºC for 1 min, an annealing step at 36ºC for 1 min, and an elongation step at 72ºC for 1.5 min. The primer extension segment was extended to 7 min at 72ºC in the final cycle.

The amplification products were resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5ug/ml) in 1X TAE buffer at 95 volts. PCR products were visualized on UV light and photographed using a GelDoc 2000 (BIORAD, USA). Amplified products were visually examined and the presence or absence of each size class was scored as 1 or 0, respectively.

Statistical analysis

Experiment was set up in randomized complete block design with eight replicates per treatment. Data were statistically analyzed using ANOVA\MANOVA of Statistica 6 software (Statsoft, 2001), the significance of differences among means was carried out using the Least Significant Test (L.S.D) at $p = 0.05$.

3. Results and Discussions

Plant growth and yields

It should be pointed out that, seed germination started simultaneously six days after sowing, and the germination rate was100% in the control as well as in the treated seeds of roselle plant during both seasons (data not shown).

In general, gamma irradiation significantly increased plant height, number of branches, number of root and root length of roselle plant as compared with control as shown in Table (2). At harvest time (132 days after sowing) 600 Gy gave the highest plant height (154.7 cm and 127.0 cm), highest number of branches (14.7 and 14.0 branches/ plant) and longest root (34.3 cm and 31.3 cm) in 2009 and 2010 seasons respectively. It can be noticed that the higher dose of gamma rays (800 Gy) was less effective than the other lower doses, seeds exposed to (800 Gy) produced the shortest plants with reduction root length compared to the other doses (Table 2). This reduction could be attributed to reduced in mitotic activity in meristematic tissues and reduced moisture content in seeds as reported by Khalil *et al.* (1986). Similary Norfadzrin *et al.* (2007) noticed that higher gamma ray doses (600 and 800 Gy) had

negative effect on the morphological characteristics of tomato and okra seedlings derived from irradiated seeds. A reduction in plant height and number of branches for many crops that exposed to higher gamma ray doses had already been reported by Thimmaiah *et al*. (1998), Muhammad and Afsari (2001), Al-Salhi *et al*. (2004), Yaqoob and Ahmad (2003), Token *et al*. (2005) and Kon *et al*. (2007).

Fresh and dry weights of plant

The fresh and dry weight of leaves stems and roots of roselle plant were significantly increased as result of gamma ray compared with control in the two seasons as shown in Table (3). The maximum values of fresh and dry weight were obtained by 600 Gy in the first season and by 500 or 600Gy in the second one. Similer trend have been reported by Veeresh *et al*., (1995) and Kon *et al.,* (2007) on beans. Abo-EI-Seoud *et al*. (1994) assumed the stimulation of gamma radiation to its impact on the auxins balance within the plant tissues. The reduction in fresh and dry weight of plant may be due to reduce moisture content due to radiation stress, when exposed to high gamma radiation doses.

The calyx yield

The application of 600 Gy gave the highest effect on increasing number of fruit per plant (Table4) compared with the other radiation doses and the control. The significantly higher calyx fresh weight per plant recorded by the application of 500 and 400 Gy (171.8 and 151.4 g per plant) in 2009 and 2010 respectively at harvest stage 180 days after sowing (Table 4). Increased growth of plants (plant height, number of branches, root length, fresh and dry weight of leave, stems and roots) as result of exposed to 600 Gy hence higher yields (fruit yield) of roselle plant (Tables 2and3). The stimulatory effect of 600 Gy dose is due to the fact that mutagens stimulate the role of enzyme and growth hormone responsible for growth and yield. Increased number of fruits per plant as a result of gamma irradiation was recorded by (Dubey *et al*., 2007; Mishra *et a*l., 2007; Sharma and Mishra, 2007 Sujaya-Das *et al*., 2007 and Sundaravadivelu *et al*., 2006).

Photosynthetic pigment

Data presented in Table (5) indicated that all photosynthetic pigment contents were significantly increased as a result of gamma irradiation increased except 300and 400 Gy. In the two seasons, best results were obtained by using 700 Gy. Higher doses of mutagens were most effective to produce chlorophyll mutations in roselle which consequently increased all yield-related traits. These results are in agreement with those obtained by (Rasico *et al*., 2001; Osama, 2002 and Rejili *et al*., 2008), who reported that the improvement of yield components and chlorophyll parameters in plants was induced after various mutagenic treatments such as E.M.S, sodium azide and gamma rays.

Anthocyanine content

The concentrations of anthocyanin are given in Fig. (1). Results indicated that anthocyanin content of roselle calyxs were increased by increasing the dose of gamma irradiation treatments when compared with control treatment. Moreover, at harvest (180 days after sowing), the 600 Gy was the most effective treatment for increasing anthocyanin which gave 3.63% and 3.68% in 2009 and 2010 respectively. These results agree with those reported by *Abo-EI-Seoud, et al*. (1994) who found that the 40 Gy had the capacity to enhance anthocyanin concentrations. Gamma rays belong to ionizing radiation and interact on atoms or molecules 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 depending on the irradiation level (Kim *et al*., 2004, Wi *et al*., 2005).

RAPD-PCR of genomic DNA

RAPD-PCR was used for detection of DNA profile changes due to treatments (0.0, 100,200,300,400,500,600,700 and 800 Gys). Six primers out of twenty-eight random 10-mer primers (OPA-16, OPB-02, OPD-02, OPD-07, OPG-10 and OPH-05) successfully amplified DNA fragments from *Hibiscus sabdariffa* L DNA samples (Table1and Fig2). The results indicated occurrence of structural changes in treatment with six primers (Table 6, Fig2). A total of 54 fragments were visualized across the six primers (Table 6). Genomic DNA polymorphisms due to treatments are presented in Figure (2). The percentage of polymorphism was (38.4, 70, 50, 77.3, 54.5 and 38.4%) (Table 6). The result of RAPD analysis indicted the disappearance of DNA polymorphic bands in response to treatments with doses of all gamma irradiation**.** In primer OPD-07 bands with molecular size 1651 bp disappeared under the effect of gamma irradiation. Bands with molecular size 1207, 322, 506 and 370 bp appeared under the effect of gamma irradiation, the first appeared only at high doses (200, 300 and 500 Gy). Bands with molecular size 370 bp appeared only at high doses (800 Gy). In primer OPA-16, band with molecular size 1234bp appeared under irradiation by 700 and 800 Gy. The bands with molecular sizes 442bp appeared only under irradiation by 300, 500, 700 and 800 Gy in primer OPD-02 (Fig2).

The results agreed with Wendt *et al*. (2001) who used the RAPD markers to study the effect of gamma radiation on potato. Ganapathi *et a*l. (2008) studied the effect of gamma irradiation on banana using RAPD-DNA analysis. They observed changes in the DNA bands, where the main changes in the RAPD profiles of the present investigation were the appearance or disappearance of different bands with variation in their intensity. These effects might be due to the structural rearrangements in DNA caused by different types of DNA damages. Appearance of new bands is usually result from different DNA structural changes (Breaks, transpositions, deletion etc) (Danylchenko and Sorochinsky, 2005).

Conclusion

The results of the experiment indicated that increasing doses of gamma irradiation caused severe effects on the plant development. In general, according to the results of the present work, the best treatment was the application of 600 Gy irradiation which stimulate roselle plant growth and, intern, to increase its active substances productivity. The ultimate aim of a mutagenic treatment is to induce mutations leading to genetic improvement of a specific trait and selection of economically important mutants. For breeding purposes mutagenic treatments with low physiological effects and strong genetic effects are desirable.

Table (2) Effect of gamma irradiation on growth criteria of *roselle* plants during 2009 and 2010 seasons.

Treatments	Plant height (cm)	Number of branches/ plant	Number of roots/ plant	Length of the Longest root (cm)
		1st season		
Control	78.3d*	6.3c	3.7e	19.0d
γ 100Gy	106.3c	10.6abc	12.0a	22.0bcd
γ 200 Gy	107.3c	12.7ab	5.7cde	26.0bc
γ 300 Gy	131.3b	6.7c	5.3de	21.3cd
γ 400 Gy	123.3b	13.7a	10.0ab	21.1cd
γ 500 Gy	121.5b	14.3a	6.3cd	27.3b
γ 600 Gy	154.7a	14.7a	9.0b	34.3a
γ 700 Gy	130.0b	10.3abc	8.0bc	22.3bcd
γ 800 Gy	87.0d	8.3bc	3.8e	21.0cd
		2nd season		
Control	85.7cd	6.0d	5.3d	15.0d
γ 100Gy	108.0b	6.7cd	13.5a	24.0bc
γ 200 Gy	108.3b	13.7ab	6.3bcd	22.3bc
γ 300 Gy	126.3a	10.7abc	7.3bc	25.0bc
γ 400 Gy	126.7a	9.3bcd	6.7bcd	25.3abc
γ 500 Gy	109.7b	10.0abcd	6.5bcd	26.7ab
γ 600 Gy	127.0a	14.0a	7.3bc	31.3a
γ 700 Gy	99.0bc	12.7ab	6.1cd	24.3bc
γ 800 Gy	78.3d	12.3ab	6.0cd	19.7cd

* Means followed by the same letter within a column are not significantly different at 0.05 level of probability accor ding to L.S.D. test

Table (3) Effect of gamma radiation on fresh and dry weight g / of leaves, stem and root of roselle plants during 2009 and 2010 seasons.

Treatments	Leaves fresh weight/ plant (g)	Leaves dry weight/ plant (g)	Stem fresh weight/ plant (g)	Stem dry weight/ plant (g)	Roots fresh weight/ plant (g)	Roots dry weight/ plant (g)
1st season						
Control	75.7de*	12.6d	64.9f	20.3d	8.1e	3.1d
γ 100Gy	87.8de	13.6cd	94.8e	36.1c	9.6de	4.1cd
γ 200 Gy	92.8de	16.8cd	75.4f	22.9d	18.0ab	5.5abc
γ 300 Gy	101.9d	24.9b	112.2d	34.3c	12.5cde	4.1cd
γ 400 Gy	91.8de	18.9c	113.9d	34.6c	13.6bcd	5.2bc
γ 500 Gy	148.5c	27.0b	212.1d	52.3b	14.2abc	5.1bc
γ 600 Gy	214.2a	36.4a	218.3a	70.6a	18.8a	6.9a
γ 700 Gy	174.1b	36.2a	200.7b	50.6b	14.7abc	6.1ab
γ 800 Gy	83.6de	12.7d	141.5c	25.3d	8.4e	4.8bc
2nd season						
Control	63.6c	11.6f	59.0g	17.8d	8.6c	2.5e
γ 100Gy	88.5c	11.8f	107.2e	31.1c	8.8c	4.2bcd
γ 200 Gy	92.4bc	17.5de	76.4f	20.5d	9.4c	3.7cde
γ 300 Gy	98.2bc	20.6cd	128.0cd	37.5b	14.5b	5.1bc
γ 400 Gy	86.1c	15.0ef	124.8d	31.3c	15.9b	5.3b
γ 500 Gy	166.6a	34.6a	164.3b	42.0a	9.4c	4.0bcde
γ 600 Gy	153.5a	30.3b	198.8a	42.2a	26.0a	8.2a
γ 700 Gy	131.1ab	22.9c	141.5c	41.7a	23.5a	7.8a
γ 800 Gy	90.4c	17.6cd	130.2cd	40.4ab	8.7c	2.6de

* Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to L.S.D. test

Table (4) Effect of gamma irradiation on some yield components in roselle plants during 2009 and 2010 seasons.

Treatments	No. of fruits / plant	Calyx weight	
		Fresh (g/plant)	Dry (g/plant)
1st season			
Control	31.7e*	70.7b	8.8e
γ 100Gy	43.0de	68.8b	8.5e
γ 200 Gy	40.0de	60.4b	8.0e
γ 300 Gy	42.0de	130.5ab	12.0cde
γ 400 Gy	86.0b	151.4ab	13.9bcd
γ 500 Gy	53.0cd	171.8a	22.4a
γ 600 Gy	113.5a	103.7ab	16.4b
γ 700 Gy	66.3c	90.7ab	15.7bc
γ 800 Gy	46.7d	73.8b	10.4de
2nd season			
Control	33.0d	80.3ab	12.4abcd
γ 100Gy	36.0d	68.7b	9.9bcd
γ 200 Gy	43.3cd	89.7ab	13.4abcd
γ 300 Gy	49.3bc	90.3ab	14.4abcd
γ 400 Gy	58.7ab	151.4a	17.2a
γ 500 Gy	50.0bc	108.5ab	16.0ab
γ 600 Gy	61.0a	84.5ab	9.9bcd
γ 700 Gy	56.7ab	109.9ab	8.6cd
γ 800 Gy	38.3d	53.9ab	7.2d

* Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to L.S.D. test

Table (5) Effect of gamma irradiation on chlorophyll (mg/100 g F.W) of roselle plants during 2009 and 2010 seasons.

Treatments	Chlorophyll "a"	Chlorophyll "b"	Chlorophyll "a+b"
1st season			
Control	47.7b	42.9ab	90.6b
γ 100Gy	47.8b	52.6ab	100.4ab
γ 200 Gy	68.1a	51.5ab	119.6ab
γ 300 Gy	70.2a	25.2b	95.8ab
γ 400 Gy	62.8a	48.3ab	110.9ab
γ 500 Gy	65.2a	46.4ab	111.6ab
γ 600 Gy	68.5a	57.6ab	126.1ab
γ 700 Gy	70.7a	69.4a	139.6a
γ 800 Gy	68.6a	71.8a	140.3a
2nd season			
Control	70.2a	23.5b	94.0c
γ 100Gy	64.4ab	44.5ab	108.9abc
γ 200 Gy	59.4ab	40.9ab	100.4bc
γ 300 Gy	65.5ab	40.8ab	106.3abc
γ 400 Gy	54.8b	39.2ab	94.0c
γ 500 Gy	61.7ab	52.1ab	113.8abc
γ 600 Gy	67.2ab	60.4a	127.6ab
γ 700 Gy	70.5a	62.1a	132.3a
γ 800 Gy	57.2ab	35.4ab	92.6c

* Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to L.S.D. test

Table (6) DNA polymorphism using randomly amplifying DNA (RAPD) for *roselle* plants during 2009 and 2010 seasons.

Primer	Total # of amplicons	Monomorphic amplicons	Polymorphic amplicons	% of polymorphism
OPA-16	13	8	5	38.4
OPB-02	10	3	7	70
OPD-02	10	5	5	50
OPD-07	15	4	11	77.3
OPG-10	11	5	6	54.5
OPH-05	13	8	5	38.4
Total	72	33	39	54
Average	12	5.5	6.5	

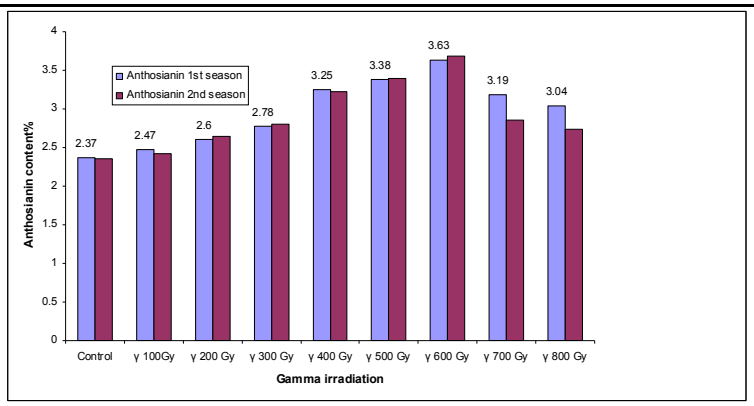

Fig (1) Effect of gamma irradiation on total anthocyanin content (%) of roselle plants during seasons 2009 and 2010.

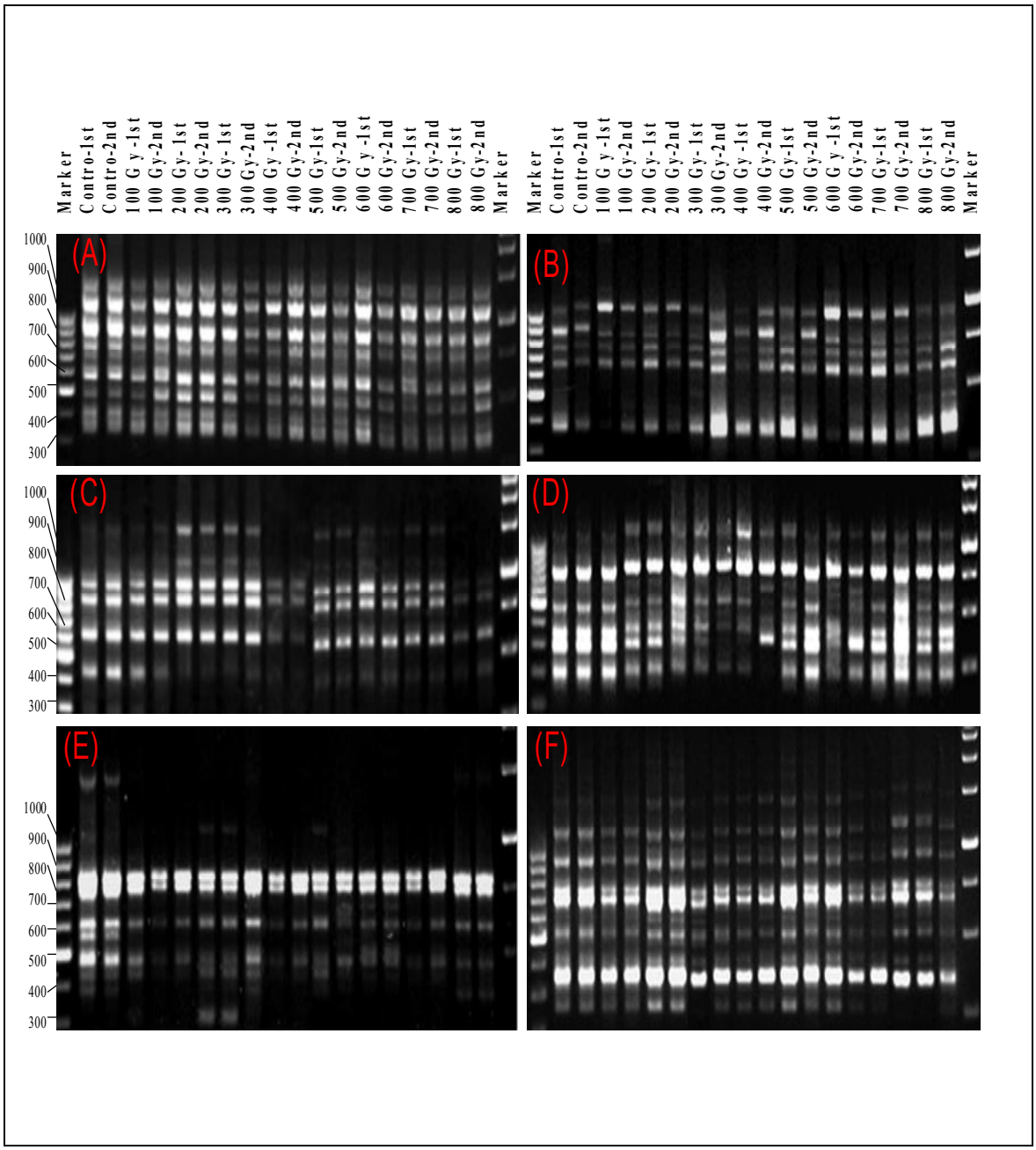

Fig. (2) DNA polymorphism using randomly amplifying DNA (RAPD) for *Hibiscus sabdariffa* treated with different doses of gamma irradiation during seasons 2009 and 2010. (A), (B), (C), (D), (E) and (F) refer to primers OPH-05, OPD-02, OPG-10, OPB-02, OPD-07 and OPA-16 respectively.

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