J. Product. & Dev., 25(1): 79-99 (2020)

GENETIC DIVERSITY AND RELATIONSHIPS AMONG GRAPEVINE ROOTSTOCK MUTANTS THROUGH RAPD TECHNIQUE

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ABSTRACT

Mutation using radiation has been shown to be an important tool in incorporating specific desirable agronomic value characteristics. In the present study, four grapevine rootstocks namely: Freedom, Harmony, SO4 and Ramsey along with commercial variety Thompson seedless were exposed to different doses of gamma rays (0, 10, 20 Gy) to obtain mutations with economic values.

***Results showed that** both doses of 10 and 20 Gy were effective in obtaining mutations in all grapevine genotypes including Thompson seedless, which were confirmed by morphological markers such as bud burst percentage and shoot length and number of leaves, and genetic markers by RAPD technique.*

In addition, genetic variation and relationships in both irradiated and un-irradiated genotypes were determined. Six RAPD markers were able to generate polymorphic bands ranged from 1 to 9 among irradiated and un-irradiated grapevine genotypes. The similarity coefficients detected by RAPD markers ranged from 0.24 to 0.87, which revealed clear genetic variation among irradiated and un-irradiated grapevine genotypes. According to phylogenetic analysis, genotypes were divided into two clusters, where all five un-irradiated genotypes and irradiated Freedom genotype with 10 Gy were placed in one cluster and second cluster included the rest of nine irradiated genotypes.

***Conclusively,** this study supports the use of mutation in breeding grapevine improvement, and RAPD technique could be an important tool for detecting mutations and genetic diversity among grapevine genotypes.*

Key words: Mutation, grapevine varieties, RAPD technique, rootstocks

INTRODUCTION

Grapes (*Vitis vinifera* L.) belong to the family Vitaceae, and rank first among fruit crops in the world in terms of the production and economic importance (Vivier and Pretorius, 2002). Grapes areas have recently increased rapidly in Egypt and reached about 77895 Hektar, which produced about 1703394 tons in 2017 season (FAO, 2017). Mutation breeding is one of the oldest breeding programs. Natural mutations and traditional breeding methods have been used to develop new genotypes that are superior with respect to some agronomic characteristics, high quality and more resistant to biotic and abiotic stress. Since natural mutations appear spontaneously, and are not so easily detectable, so many researchers have used artificial mutations in different plant varieties to induce genetic variations, which are easier, cheaper and more variable (Donini, 1993).

Selection of appropriate genotypes from positive or negative variations occurred by different mutagen doses is the main principle of mutation breeding. In general, vegetative and generative parts of plants such as cutting, tuber, stem, seed etc. are used in mutation applications. Breeding mutations program has become more common and widespread among plant breeders in particularly with the limitations of some biotechnologies in Egypt such as wide hybridizations and genetically modified plants. It is of great importance in determination of suitable mutagen doses, which have lower physiological damage and enable to provide us higher genetic effect and variations (Klu and Haarlent, 2000).

Nowadays, the number of cultivars derived from mutation induction increases constantly (Hearn, 2001). Inducing mutations by gamma rays has been effectively used with several species. Irradiation of gamma rays on bud wood can produce higher frequencies of mutation leading to the creation of new genetic recombinations. Random Amplified Polymorphic DNA (RAPD) markers are suitable to perform with good polymorphism and can be used in examining genetic diversity and the relation between species at molecular level (Lanying *et al*. 2008, Arya *et al*. 2010). It consists of fragments having 10 nucleotides in length which are amplified through PCR of random segments of genomic DNA with one primer of random nucleotide sequence. RAPDs have been used consistently as molecular markers for classification of different grapes cultivars (Buscher *et al*., 1993).

Therefore, this study was aimed to induce mutations by gamma radiation in different grapevine genotypes, and to detect genetic diversity and relationships among mutants produced through RAPD technique for development of pre-breeding abiotic-tolerant grapevine genotypes which could be used as rootstocks in Egypt.

MATERIALS AND METHODS

The present study was conducted during two consecutive experimental seasons (2015 and 2016) at the experimental farm of Faculty of Agriculture, Kafrelsheikh University, Egypt.

1- ***Plant materials***

Four grapevine rootstocks were used in the present study namely: Freedom (1613C x *V. champini*), Harmony (1613C x *V.s champini*), SO4 (*V. berlandieri* x *V. riparia*), Ramsey (*V. candicans* x *V. rupestris*), and grapevine cultivar "Thompson seedless" (*V. vinifera* L.). These genotypes were obtained from El-Roda farm located at Beheira Governorate.

2- ***Gamma irradiation doses and data recorded***

Forty-five cuttings from each selected grape rootstock along with grapevine variety "Thompson seedless" were cut and collected on the 31th of January 2015 and 2016. The selected cuttings with three buds/cutting and length at 20cm were irradiated with five doses of gamma rays 0, 10, 20, 30 and 50 Gy. Gamma rays were generated using cobalt 60 as a source of gamma rays. Irradiation of the cuttings was carried out at the National Center for Radiation Res. and Tech., Nasr City, Cairo, Egypt.

In the first week of February in both seasons (2015 and 2016), the irradiated cuttings were individually planted in plastic pots (35 cm. in diameter) each filled with approximately 6 Kg of soil mixture from clay and sand (1:1 v/v). The cuttings in the pots were irrigated with tap water weekly at the rate of one liter/pot till the investigate treatments started on the 1st April of 2015 and 2016. All pots were supplied with a complex N, P, K Fertilizers (1:1:1) as well as Fe, Mn and Zn micro elements in chelated form.

The following data was recorded

1- Vegetative growth traits

Vegetative growth traits were measured on irradiated cuttings of grapevine rootstocks and Thompson seedless as described below.

A- Bud burst percentage (opening bud %): Number of bud burst, was calculated according to Huglin (1958).

$$\text{Bud burst \%} = \frac{\text{No. of bursted buds/vine}}{\text{Total buds/vine}} x100$$

B- Shoot length: Average shoot length (cm) was measured by ruler

C- Number of leaves/plant: All new leaves developed on these shoots were counted.

D- Survival percentage: it was calculated using the following formula.

$$\text{Survival \%} = \frac{\text{Total No. of cuttings - Total No. of obening cuttings}}{\text{Total No. of cuttings}} x100$$

3. *RAPD analysis*

RAPD analysis was carried out in Genetic Engineering and Tissue Culture Laboratory (GETCL) at faculty of Agriculture, Kafrelsheikh University. PCR analysis was carried out on five grape genotypes (unirradiated "control" and irradiated cuttings at two doses 10 and 20 Gy, since the dose 30 Gy was not available for DNA isolation.

A- *DNA isolation*

DNA was isolated using CTAB method from fresh leaves of five grapes rootstocks and their radiated with doses 10 and 20 Gy according to Doyle and Doyle (1990). Fresh 100-150 mg of young leaves was collected and powdered under liquid nitrogen using mortar and pestle. The ground material was transferred into Eppendorf tubes. The 800 µl of CTAB extraction buffer were added followed by vortexing. The tubes were incubated for 30 min. at 60°C. After incubation, 800 µl CI-mix were added and tubes were gently mixed by inverting the tube 4-5 times to avoid shearing of genomic DNA. The mixture was centrifuged at room temperature for 10 min. at 12000 rpm. The aqueous phase (app. 800 µl) was transferred into a fresh 1.5 ml Eppendorf tube. The centrifugation step was repeated to get a clear sample. About 550 µl of pre-cooled isopropanol were added. The tubes were centrifuged for 10 min. at 14000 rpm to precipitate the genomic DNA. The supernatant was discarded and the DNA pellet was washed with 200 µl washing buffer until the pellet floats. Washing buffer was carefully removed and the pellet was resuspended in 200 µl TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) supplemented with RNase A (10 µg/ml). The sample was incubated for 30 min. at 37°C. 100 µl of 7.5 M NH4-acetate and 750 µlabsolute ethanol were added and gently mixed. The mixture was centrifuged at maximum speed for 10 min. at room-temperature. The supernatant was discarded completely and the pellet was dried for 40-50 min. at 37°C. After drying, the pellet was re-suspended in 100 µl TE buffer. DNA concentration and purity was assessed using Nanodrop-spectrophotometer (IMPLN Germany).

The isolated DNA samples were amplified using Taq DNA Polymerase 2x-preMix kit following the manufacturer protocol (GeneON, # S113 and Taq DNA Polymerase 2X-preMix) and PCR Techne (TC-3000, USA). Eight multiplexing sets of RAPD primers were used in this study (Table 1). The PCR reactions were optimized and mixtures were prepared (in total volume of 25 µl).

Table (1): List of multiplexing sets of the used RAPD primers in the present study

No.	Primer name	primer seq.,
1	OPA-04	AATCGGGCTG
2	OPA-05	AGGGGTCTTG
3	OPA-06	GGTCCCTGAC
4	OPA-10	GTGATCGCAG
5	OPA-11	CAATCGCCGT
6	OPA-12	TCGGCGATAG
7	OPA-13	CAGCACCCAC
8	OPA-14	TCTGTGCTGG

PCR cycling was carried out as the following program, one cycle at 95 °C for 5 min., and then 35 cycles were performed as follows: 1 min. at 95 °C for denaturation, 1 min. at 36°C for annealing and 1.30 min. at 72 °C for extension.

Reaction was incubated at 72°C for 7 min. and then kept at 4°C. The loading buffer (5 µl) was added to each sample of PCR product (15 µl).

PCR products were separated by electrophoresis using 1% agarose gel in 0.5 x TBE buffer against 100 bp DNA Ladder as a size marker. Bands were detected with ethidium bromide staining and documented on Gel Documentation UVITEC, UK.

The run was performed for one hour at 80 volts in Bio-Rad submarine (8 cm x 12 cm). Agarose Gel electrophoresis was used to detect DNA fragments according to (Buitkamp *et al.,* 1991) to determine the size of the PCR products.

3- ***Statistical analysis***

All data about vegetative growth and molecular analysis were expressed as means. The statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS, 18.0 software (SPSS, 2011).

The individual comparisons were obtained by Duncan's multiple range test (DMRT). Values were considered statistically significant when $P < 0.05$.

RESULTS AND DISCUSSION

1- *Vegetative growth*

A. Opening buds (%)

The results presented in Tables (2 and 3) showed that percentage of opening buds was increased significantly with unirradiated Thompson seedless

variety (control) and Thompson seedless irradiated with 10 Gy of gamma rays. Meanwhile the lowest percentage was observed in Freedom variety with 20 Gy at all sampling dates during 2015 and 2016 seasons. Thompson seedless cultivar recorded the highest percentage of opening bud followed by So4, Ramsey, Harmony and Freedom in both seasons.

The highest opening buds % obtained by the control treatment (9.33 and 9.60%) followed by genotypes irradiated with gamma rays at 10 Gy and the lowest values was observed with genotypes irradiated with dose 20Gy (5.07 and 5.27%) in the two seasons respectively. These results suggest that gamma rays with high doses cause growth inhibition of buds which might be ascribed to the cell cycle arrest during somatic cell division and /or various damages in the entire genome (Preussa and Britta, 2003), also the reduction may be due to high content of ABA (Rabie *et al.*, 1996), destructions or damage of apical meristem or reduction in the level of amylase activity and to temporary suspension of cell division or delay in mitosis or increase in the production of active radicals (Esmault *et al.*, 2010). These results are in agreement with those obtained by previous studies (Coban *et al*., 2002, Tayyer *et al*., 2003, Dardeniz and Tayyer.,2005).

B. *Shoot length (cm)*

There was significant difference in the shoot length between the combination treatments in both seasons (Tables 4 and 5). As the growth progressed, the maximum shoot length was obtained with un-irradiated Thompson seedless and genotypes irradiated with 10 Gy . Conversely, the lowest length of shoot was recorded with the combined treatments of Freedom x 20 Gy at all sampling dates during 2015 and 2016 seasons.

The highest shoot length recorded by Thompson seedless cultivar (Tables 4 and 5). Regarding to the effect of gamma ray doses, there were no significant differences among all doses of gamma rays in both seasons. The results obtained in the present investigation are similar to those of (Saiful *et al*.,2015, Tayyer *et al*.,2003, Dardeniz *et al.*,2005, Surakshitha *et al*.,2017).

C. *Number of leaves*

The combined treatments of Thompson seedless with no irradiation and Thompson seedless irradiated with 10 Gy gave the highest number of leaves as compared with the lowest number obtained by the combined treatments of Freedom with no irradiation, Freedom x 10 Gy and Freedom x 20Gy in the first season (Table 6).

Table (2): Opening bud percentages in un-irradiated and irradiated grapevine during 2015 season

Treatments \ Months		16/2	23/2	2/3	9/3	16/3	23/3	30/3	6/4
		Season, 2015							
A. Interaction: Root stocks X G. ray									
Thompson seedless	Cont.	32ab	44.00a	52.00a	56.00a	65.33a	69.33a	72.00a	69.33a
	10	12.00b	40.00ab	48.00ab	52.00ab	65.33a	64.00a	66.67a	66.66a
	20	6.67cd	36.00bc	44.00abc	52.00ab	62.67a	60.67a	64.00a	64.00a
SO4	Cont.	6.67cd	36.00bc	40.00bcd	48.00abc	50.67b	49.33b	52.00b	53.33b
	10	8.00bcd	30.67cd	38.67bcd	41.33bcd	49.33bc	45.33bc	52.00b	52.00b
	20	6.67cd	29.33d	37.33bcd	40.00cd	45.33bcd	45.33bc	48.00bc	49.33bc
Ramsey	Cont.	4.00de	22.67e	37.33bcd	38.66cd	41.33bcde	44.00bc	48.00bc	46.67bcd
	10	5.33d	22.67e	34.67cd	36.00cd	40.00bcde	40.00bcd	44.00bc	46.67bcd
	20	8.00cd	22.67e	34.67cd	36.00cd	38.67bcde	38.33bcd	40.00bc	42.67cd
Harmony	Cont.	4.00de	21.33e	33.33cd	36.00cd	38.67bcde	37.33bcd	40.00bc	40.00cd
	10	10.67bc	20.00e	33.33cd	36.00cd	36.00cde	37.33bcd	38.67bc	40.00cd
	20	4.00de	20.00e	32.00cd	34.67cd	34.67de	34.67cd	36.00c	38.67d
Freedom	Cont.	1.00e	20.00e	32.00cd	33.33d	33.33de	34.67cd	32.00c	38.67d
	10	5.33d	20.00e	32.00cd	32.00d	29.33e	34.67cd	32.00c	37.33d
	20	1.00e	20.00e	28.00d	32.00d	20.00f	29.33d	32.00c	37.33d
Grapevine rootstocks									
Thompson seedless		16.88a	38.67a	46.22a	48.00a	53.78a	54.00a	58.22a	57.78a
So4		7.11b	29.78b	36.44b	44.00a	48.44ab	49.78a	50.67b	50.67b
Ramsey		5.78b	24.44c	34.67b	38.67b	43.56bc	39.89b	46.22c	46.22c
Harmony		6.22b	21.33c	34.22b	35.56b	38.22cd	39.56b	41.33d	46.22c
Freedom		1.78c	20.89c	34.22b	35.11b	32.89d	38.22b	36.00e	40.00d
Gamma ray									
Cont.		9.33a	29.86a	40.53a	42.13a	50.67a	49.73a	51.20a	51.47a
10		8.27a	27.46b	36.00b	39.73a	40.27b	45.07b	48.53a	51.20a
20		5.07b	23.73c	34.93b	38.93a	39.20b	38.07c	39.73b	41.87b

Table (3): Opening bud percentages in un-irradiated and irradiated grapevine rootstocks during 2016 season

Months / Treatments		16/2	23/2	2/3	9/3	16/3	23/3	30/3	6/4
		Season, 2016							
A. Interaction: Rootstocks X G. ray									
Thompson seedless	Cont.	32.67a	42.00a	52.00a	55.33a	66.00a	69.00a	69.00a	70.33a
	10	10.67b	39.00a	45.67ab	53.33a	65.33a	65.67a	67.66a	64.67a
	20	7.67bcd	36.67a	44.33abc	51.00a	63.67a	62.00a	34.66cd	63.33a
SO4	Cont.	6.33bcd	35.00a	40.67bcd	46.33ab	50.33b	48.33b	66.00a	53.67b
	10	7.67bcd	28.00b	40.33bcd	41.00bc	48.67b	46.67bc	49.00b	53.67b
	20	7.00bcd	26.67bc	38.67bcd	40.33bc	45.33bc	43.67bc	41.33bcd	49.33bc
Ramsey	Cont.	4.00de	21.67bc	37.00bcd	39.00bc	41.00bc	43.67bc	40.00bcd	46.33bcd
	10	6.33bcd	20.33bc	36.67bcd	36.67bc	40.67bc	40.33bcd	32.66d	46.00bcd
	20	7.67bcd	20.33bc	34.33bcd	36.67bc	39.00bc	39.00bcd	50.66b	39.66cd
Harmony	Cont.	5.00cde	20.00bc	32.67cd	36.33bc	38.67bc	38.67bcd	33.33d	39.33cd
	10	9.33bc	20.00bc	32.00cd	36.00bc	35.33bcd	37.00bcd	43.33bcd	39.00cd
	20	4.00de	20.00bc	31.67cd	34.00bc	35.00bcd	35.67cd	33.00d	39.00cd
Freedom	Cont.	1.00e	19.00bc	31.00d	33.33c	33.33bcd	35.33cd	46.00bc	38.67cd
	10	4.67cde	18.33c	31.00d	32.00c	30.33cd	34.67cd	50.33b	38.00d
	20	1.00e	17.33c	28.33d	31.33c	21.00d	28.67d	39.33bcd	37.33d
Grapevine rootstocks									
Thompson seedless		17.00a	37.88a	46.00a	48.33a	54.33a	54.44a	57.11a	58.00a
So4		7.00b	27.67b	36.78b	43.67a	48.67ab	50.11a	52.11a	50.22b
Ramsey		6.00b	22.89c	34.56b	38.33b	42.89bc	40.44b	41.11bc	46.33b
Harmony		6.11b	20.00c	34.44b	35.56b	38.56cd	40.22b	36.55c	46.11b
Freedom		1.56c	19.67c	33.67b	35.00b	33.44d	37.56b	45.22b	38.78c
Gamma ray									
Cont.		9.6a	28.07a	40.60a	41.67a	50.67a	50.07a	50.87a	51.20a
10		7.73a	26.33a	36.73b	39.60a	40.73b	45.60b	48.60a	50.60a
20		5.27b	22.47b	33.93b	39.27a	39.33b	38.00c	39.80b	41.87b

Table (4): Means of shoot length (cm) in un-irradiated and irradiated grapevine rootstocks during 2015 season

Treatments \ Months		6/4	6/5	6/6	6/7	6/8	6/9
		Season, 2015					
A. Interaction: Root stocks X G. ray							
Thompson seedless	Cont.	11.33a	25.00a	25.67a	45.00a	58.33a	68.33a
	10	10.33ab	20.00b	20.00ab	40.00ab	58.33a	65.00ab
	20	8.00bc	13.33c	20.00ab	40.00ab	50.00ab	58.67bc
SO4	Cont.	7.00c	11.67cd	18.67bc	35.00b	50.00ab	58.33bc
	10	6.67c	11.00cd	17.67bc	35.00b	48.33b	53.33cd
	20	5.00c	9.00cd	16.00bcd	35.00b	45.00b	48.33de
Ramsey	Cont.	5.00c	8.67cd	15.67bcd	25.00c	37.67c	45.00de
	10	5.00c	8.33cd	14.67bcd	25.00c	35.00cd	45.00de
	20	5.00c	8.33cd	13.33bcd	25.00c	35.00cd	43.33de
Harmony	Cont.	4.67c	8.33cd	13.33bcd	25.00c	35.00cd	43.33de
	10	4.33c	8.00cd	13.33bcd	25.00c	30.00cd	40.00e
	20	4.00c	7.67cd	12.00bcd	20.00cd	30.00cd	30.00f
Freedom	Cont.	4.00c	7.67cd	11.00cd	18.33cd	25.33de	25.67fg
	10	4.00c	6.67d	9.00d	17.67cd	25.00de	25.00fg
	20	4.00c	5.33d	9.00d	15.00d	18.33e	18.33g
Grapevine rootstocks							
Thompson seedless		9.89a	19.44a	19.67a	33.33a	45.33a	55.67a
So4		5.33b	9.11b	16.78b	31.67ab	44.44a	52.78a
Ramsey		5.11b	9.00bc	14.56c	30.00ab	42.78a	46.67b
Harmony		4.67b	8.67bc	14.33c	28.33b	36.67b	42.78b
Freedom		4.44b	6.78c	11.11d	18.67c	24.56c	24.67c
Gamma ray							
Cont.		6.27a	12.07a	16.33a	30.67a	39.40a	46.87a
10		6.20a	10.67ab	15.13a	28.53ab	39.33a	46.00a
20		5.20a	9.07b	14.40a	26.00b	37.53a	40.67b

Table (5): Means of shoot length (cm) in un-irradiated and irradiated grapevine rootstocks during 2016 season

Treatments \ Months		6/4	6/5	6/6	6/7	6/8	6/9
		Season, 2016					
A. Interaction: Root stocks X G. ray							
Thompson seedless	Cont.	12.00a	24.83a	24.50a	43.67a	57.00a	68.67a
	10	10.33ab	19.67b	19.67ab	38.67ab	55.83a	63.00ab
	20	8.00bc	13.67c	18.50ab	38.00ab	49.17ab	58.33b
SO4	Cont.	7.67bc	12.67c	18.00ab	36.17b	48.83ab	56.83bc
	10	7.33bc	9.83c	17.83ab	34.67b	47.83ab	52.67bcd
	20	6.33bc	9.67c	15.83bc	33.33b	44.83b	47.17cde
Ramsey	Cont.	5.67c	9.67c	14.17bc	26.67c	37.83c	44.83de
	10	5.67c	9.17c	13.83bc	25.83c	35.00cd	43.67de
	20	5.33c	9.00c	12.83bc	25.17cd	34.67cd	42.33de
Harmony	Cont.	5.33c	8.67c	12.67bc	25.17cd	33.83cde	41.83de
	10	5.33c	8.17c	12.33bc	24.67cd	29.33cde	37.17ef
	20	5.00c	8.00c	11.67bc	19.67cde	28.83cde	30.00fg
Freedom	Cont.	5.00c	7.83c	11.50bc	19.50cde	25.17def	25.17gh
	10	4.00c	6.50c	9.17c	17.00de	24.00ef	23.83gh
	20	4.00c	6.00c	8.50c	15.00e	17.67f	17.33h
Grapevine rootstocks							
Thompson seedless		10.11a	19.39a	18.83a	33.61a	44.83a	54.72a
So4		6.00b	9.78b	15.28b	31.33ab	43.00a	51.67ab
Ramsey		5.89b	9.11b	14.50b	29.44b	41.94a	45.61bc
Harmony		5.56b	9.00b	14.28b	27.94b	36.11b	41.44c
Freedom		4.78b	7.17b	10.78c	18.72c	24.06c	24.17d
Gamma ray							
Cont.		6.87a	12.37a	15.73a	29.90a	38.53a	45.97a
10		6.46a	10.23b	14.63a	28.33ab	38.47a	45.10a
20		6.07a	10.07b	13.83a	26.40b	36.97a	39.50b

In the second season, the following combined treatments: Thompson seedless x 0 Gy and Ramsey x 10Gy were markedly increased the number of leaves compared with the lowest values obtained by the combined treatment of Thompson seedless x 20 Gy (Table 7). With respect to the effect of grapevine cultivars, data tabulated in Tables (6 and 7) showed that, Thompson seedless and So4 cultivars significantly increased number of leaves and Freedom cultivar produced the lowest number of leaves.

The highest number of leaves was recorded by control (0Gy) and 10Gy of gamma radiation compared with the lowest values recorded by the highest doses of gamma ray (20Gy) in the first season. In the second season, the differences were not significant at all sampling dates except at 6/5 and 6/7. The highest number of leaves obtained under control treatment (0Gy) and 10 Gy dose of gamma ray, and gamma ray with 20 Gy resulted the lowest values.

Generally, growth inhibition might be induced by high doses of irradiation and this could be due to cell cycle arrest during somatic cell division and/or to a variety of damages in the entire genome (Shah *et al.,* 2008). Processes like auxin destruction, changes of the ascorbic acid contents, and physiological and biochemical disturbances could also induce the inhibition of plant germination and development (Preussa and Britta, 2003). The frequency of chromosomal damage with increasing doses may be responsible for reduction in plant survival and development (Kiong *et al.*, 2008). Similar trend has also been reported by Tayyer *et al*., (2003), Dardeniz *et al.,* (2005), and Saiful *et al.* (2015).

D. *Survival rate (%)*

The results illustrated in Figure (1) showed that the highest percentage of plant survival recorded with Thompson seedless received no irradiation and genotypes irradiated with 10 Gy. Concerning to genotypes, the highest survival percentage obtained with Thompson seedless cultivars followed by So4, Freedom, Ramsey and Harmony in both seasons.

The reduction in survival percentage could be due to negative effects of irradiation which increases the formation of Reactive oxygen species (ROS) in plant cell causes damages in cellular homeostasis and progressive oxidative damage and finally cell death (Beyaz and Yildiz, 2016).

2- *Genetic diversity and relationships by RAPD analysis*

Eight Random Amplified Polymorphic DNA (RAPD) markers were used in the present study to detect mutations induced by gamma irradiation, and to investigate genetic diversity and relationships among four grapevine rootstocks

Table (6): Means of leaves number in un-irradiated and irradiated grapevine rootstocks during 2015 season

Treatments \ Months		6/4	6/5	6/6	6/7	6/8	6/9
		Season, 2015					
A. Interaction: Rootstocks X G. ray							
Thompson seedless	Cont.	6.00a	19.00a	25.00a	31.67bcd	35.67e	72.67a
	10	5.67ab	17.67abc	21.00ab	35.00abc	45.00c	70.00a
	20	5.00b	13.33g	19.00b	21.67d	31.67e	69.67a
SO4	Cont.	5.00b	18.00ab	25.00a	33.33bc	41.67cd	68.00a
	10	5.00b	18.00ab	24.67a	35.00abc	43.33c	62.67b
	20	5.00b	17.67abc	25.33a	35.67abc	45.67c	60.33bc
Ramsey	Cont.	4.00c	14.00fg	24.33a	22.33d	31.33e	59.00bc
	10	4.00c	19.00a	21.00ab	31.00bcd	45.00c	57.00c
	20	4.00c	18.00ab	22.33ab	37.33ab	51.33ab	56.67c
Harmony	Cont.	4.00c	17.67abc	24.33a	36.67ab	46.00bc	55.00c
	10	4.00c	17.33bcd	21.00ab	30.bcd	36.67de	50.00d
	20	4.00c	16.00de	22.33ab	29.33bcd	46.67bc	48.00d
Freedom	Cont.	3.00d	16.33cde	25.00a	38.33ab	55.00a	48.00d
	10	3.00d	15.00ef	25.00a	45.00a	53.33a	46.33d
	20	3.00d	14.00fg	19.00b	25.00cd	35.00e	42.33e
Grapevine rootstocks							
Thompson seedless		5.00a	16.67b	21.67b	29.44b	37.44c	63.56a
So4		4.89a	17.89a	25.00a	34.67ab	43.56b	60.11b
Ramsey		4.00b	17.00b	22.56ab	30.22b	42.56b	57.56bc
Harmony		4.00b	17.00b	22.56ab	32.00ab	43.11b	57.00c
Freedom		3.67b	15.11c	23.00ab	36.11a	47.78a	50.33d
Gamma ray							
Cont.		4.60a	17.00a	24.73a	32.47b	41.93b	58.00a
10		4.40a	17.40a	22.53ab	35.20a	44.67a	57.60a
20		3.93a	15.80b	21.60b	29.80c	42.07ab	57.53a

Table (7): Means of leaves number in un-irradiated and irradiated in grapevine rootstocks during 2016 season

Months / Treatments		6/4	6/5	6/6	6/7	6/8	6/9
		Season, 2016					
A. Interaction: Root stocks X G. ray							
Thompson seedless	Cont.	5.33a	18.67a	25.00a	31.00bcd	36.67fgh	71.66a
	10	5.00a	17.00abc	20.33a	35.00ab	43.67cdef	70.33a
	20	5.00a	11.67d	19.67a	21.67d	31.33h	69.00a
SO4	Cont.	5.00a	18.00ab	24.33a	32.67abcd	41.00defg	68.33a
	10	4.33a	18.33a	24.67a	33.33abcd	43.33cdef	62.33b
	20	4.33a	17.33abc	25.67a	34.67abc	45.33bcde	59.67bc
Ramsey	Cont.	4.00a	13.67bcd	23.67a	22.67cd	30.67h	58.00bc
	10	4.00a	18.67a	20.33a	30.00bcd	44.67cdef	57.33bc
	20	3.67a	18.33a	20.00a	37.33ab	50.33abc	56.67bc
Harmony	Cont.	3.67a	17.67ab	23.00a	37.33ab	46.00abcde	54.00cd
	10	3.33a	16.33abc	19.00a	29.67bcd	38.00efgh	50.00de
	20	3.33a	15.67abcd	21.00a	29.00bcd	48.67abcd	48.00de
Freedom	Cont.	3.00a	18.00ab	24.67a	37.67ab	54.33a	47.33de
	10	2.33a	14.33abcd	24.33a	44.00a	53.33ab	46.33de
	20	2.00a	13.00cd	19.00a	26.00bcd	34.67gh	42.33e
Grapevine rootstocks							
Thompson seedless		4.44a	15.78ab	21.67ab	29.22b	37.22c	63.33a
So4		4.44a	17.89a	24.89a	33.56ab	43.22b	59.78ab
Ramsey		3.78a	16.89ab	21.33ab	30.00ab	41.89b	57.44b
Harmony		3.44a	16.56ab	21.00b	32.00ab	44.22ab	56.44b
Freedom		3.33a	15.11b	22.67ab	35.89a	47.44a	50.11c
Gamma ray							
Cont.		4.07a	17.20a	24.13a	32.27b	41.73a	58.00a
10		4.00a	16.93a	21.73a	34.40a	44.60a	57.60a
20		3.60a	15.20b	21.07a	29.73c	42.07a	57.53a

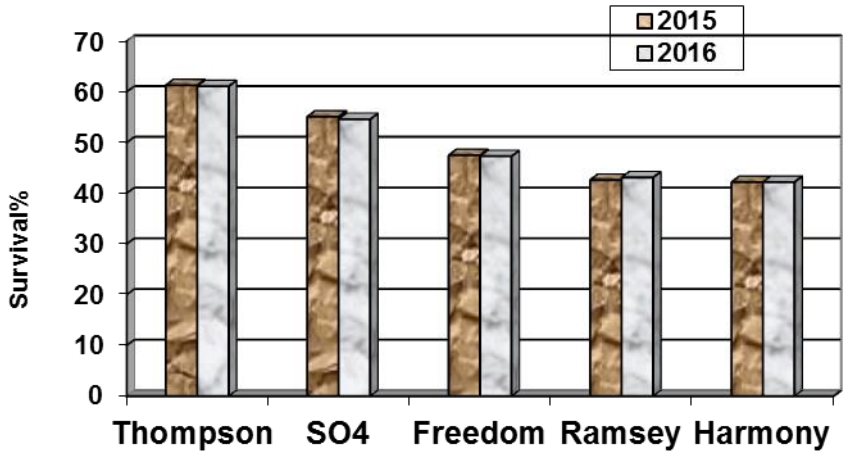


Figure (1): Survival percentage as affected by irradiated grape vine rootstocks during 2015 and 2016 seasons.

and commercial variety Thompson seedless (Table 8 and Figure 2). Among eight RAPD primers, six primers were generated polymorphic bands. No bands were detected by the primers OPA-6 and OPA-13 across the irradiated and un-irradiated grapevine genotypes. The total number of bands generated per primer varied from 1 to 9.

The primer OPA-10 in SO4 genotype showed the lowest number of bands, while the highest numbers of bands were scored in Harmony genotype using the same primer (OPA-10). Although there were no accession-specific markers used in the present study, the high level of polymorphism suggests that RAPD markers could be considered a useful tool for detecting genetic diversity among different grapevine genotypes.

In general, total and polymorphic bands were higher in un-irradiated genotypes followed by genotypes irradiated with 10 Gy. The numbers of polymorphic bands were ranged from 1 to 9, 1 to 7 and 0 to 5 in un-irradiated genotypes, and irradiated genotypes with 10 Gy and 20 Gy, respectively. The highest numbers of polymorphic bands were observed in OPA-10 in both irradiated and non-irradiated genotypes where all bands generated by this primer were polymorphic. The genetic similarity based on six RAPD primers ranged from 0.24 to 0.87 (Table 8). The highest genetic similarity 87% was found between unirradiated genotypes Harmony and Ramsey, on the other

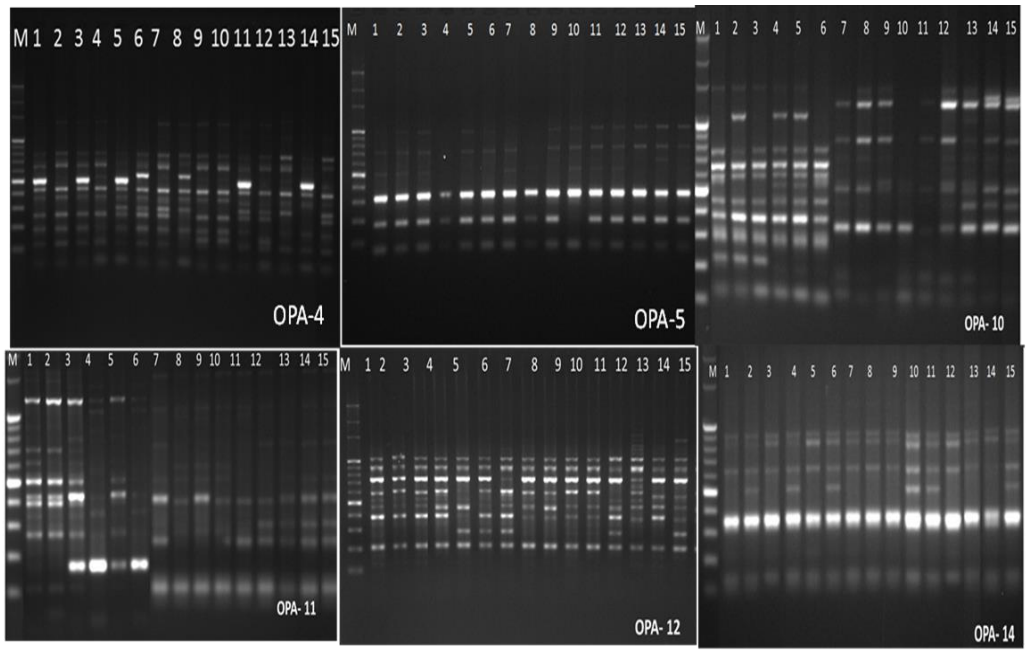


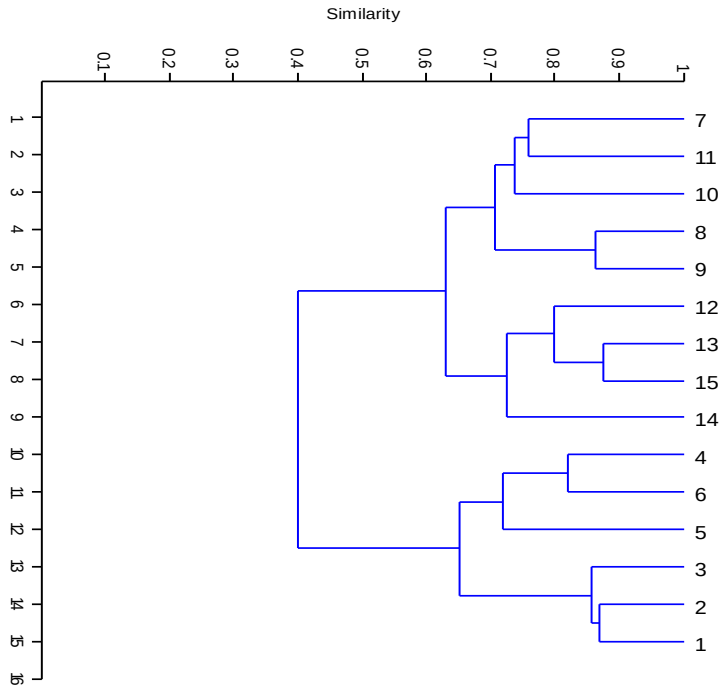
Figure (2). Survey of RAPD markers selected to detect induced mutation in Freedom, Harmony Ramsey, Thompson Seedless and So4 for primers (OPA-04, OPA-05,OPA-10,OPA-11,OPA-12 ,OPA-

hand, the lowest genetic similarity 24% was found between irradiated and un-irradiated Thompson seedless genotype. The obtained results in Table 8 showed that the similarity indices were increased as the dose of gamma rays were increased in most of grapevine genotypes. Previous studies have also been shown RAPD markers to be efficient in distinguishing between grapevine rootstocks, and advantageous since it is cheaper and easier to perform than RFLP analysis or isoenzyme characterization.

The dendrogram (Figure 3) indicated two main clusters, one comprising un-irradiated five genotypes (Freedom, Harmony, SO4, Ramsey, and Thompson seedless) and irradiated Freedom with 10 Gy, while the second cluster included the rest of irradiated genotypes indicating their higher genetic distinctness between irradiated and un-irradiated genotypes. According to the dendrogram obtained, irradiated genotypes Freedom with 20 Gy and Harmony with 10 Gy were more distant compared to un-irradiated genotypes. In addition, results showed that un-irradiated So4 and Freedom genotype with 10 Gy were the closest from Thompson seedless variety in un-irradiated and irradiated genotypes, respectively.

Table (8). RAPD profiles using selected to detect induced mutation in Freedom, Harmony, Ramsey, Thompson Seedless and So4 for primers (OPA-04, OPA-05, OPA-10, OPA-11, OPA-12, OPA-14)

Factors			RAPD markers																	
			OPA-04			OPA-05			OPA-10			OPA-11			OPA-12			OPA-14		
Rootstocks	Gamma ray (Gy)	Sample code	TB	MB	PB	TB	MB	PB	TB	MB	PB	TB	MB	PB	TB	MB	PB	TB	MB	PB
Freedom	0	1	8	1	7	4	3	1	8	0	8	6	0	6	6	3	3	3	2	1
Harmony	0	2	7	1	6	5	3	2	9	0	9	5	0	5	6	3	3	4	2	2
Ramsey	0	3	8	1	7	5	3	2	8	0	8	4	0	4	7	3	4	4	2	2
Thompson seedless	0	4	8	1	7	3	3	0	7	0	7	2	0	2	7	3	4	5	2	3
SO4	0	5	6	1	5	4	3	1	7	0	7	4	0	4	6	3	3	4	2	2
Freedom	10	6	8	1	7	4	3	1	6	0	6	1	0	1	6	3	3	5	2	3
Harmony	10	7	6	1	5	3	3	0	4	0	4	3	0	3	6	3	3	4	2	2
Ramsey	10	8	6	1	5	3	3	0	5	0	5	2	0	2	6	3	3	4	2	2
Thompson seedless	10	9	6	1	5	5	3	2	5	0	5	2	0	2	6	3	3	4	2	2
SO4	10	10	6	1	5	5	3	2	1	0	1	2	0	2	5	3	2	5	2	3
Freedom	20	11	5	1	4	5	3	2	4	0	4	2	0	2	5	3	2	4	2	2
Harmony	20	12	3	1	2	5	3	2	5	0	5	3	0	3	5	3	2	5	2	3
Ramsey	20	13	3	1	2	5	3	2	3	0	3	3	0	3	5	3	2	3	2	1
Thompson seedless	20	14	2	1	1	5	3	2	4	0	4	3	0	3	5	3	2	2	2	0
SO4	20	15	3	1	2	5	3	2	4	0	4	3	0	3	4	3	1	4	2	2



. **Figure (3).** Genetic relaetness among grapevine rootstocks treated with gamma rays

Dendrogram showing the genetic relatedness of five grapevine rootstocks along with a commercial scion Thompson seedless cultivar treated with gamma rays through six RAPD primers. 1=cont Freedom 2=cont Harmony 3= cont Ramsey 4= cont Thompson seedless 5= cont So4 6= 10 GY Freedom 7=10GY Harmony 8=10GY Ramsey 9=10GY Thompson seedless 10=10GY SO4 11=20GY Freedom 12= 20GY Harmony 13=20GY Ramsey 14=20Gy Thompson seedless 15= 20GY So4.

CONCLUSION

Radiation is one of the most successful mutagens used in plant breeding programs. It has the ability to change the plant genome and increase genetic variability in a short time in a safe and conclusive manner. Hence, the plant breeder can make the selections with desirable traits. This might be helpful in grapevine rootstocks for selecting genotypes with high tolerance to biotic and abiotic stress. In the present study, mutations were induced through gamma irradiation (10 and 20 Gy) in five grapevine genotypes including commercial variety Thompson seedless. These mutations were confirmed by morphological

Table (9): Similarity matrix for grapevine rootstocks through six RAPD primers based on Jaccard analysis

	2	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
1	0.87	0.87	0.63	0.61	0.58	0.39	0.39	0.43	0.40	0.36	0.33	0.36	0.28	0.32
2	1.00	0.85	0.70	0.63	0.64	0.38	0.38	0.45	0.46	0.36	0.38	0.38	0.27	0.37
3		1.00	0.70	0.72	0.64	0.41	0.44	0.52	0.46	0.42	0.38	0.38	0.30	0.34
4			1.00	0.75	0.82	0.53	0.45	0.46	0.51	0.39	0.38	0.35	0.24	0.34
5				1.00	0.69	0.43	0.39	0.40	0.41	0.40	0.36	0.33	0.31	0.29
6					1.00	0.59	0.42	0.47	0.63	0.47	0.46	0.43	0.30	0.42
7						1.00	0.73	0.74	0.72	0.76	0.68	0.66	0.53	0.63
8							1.00	0.86	0.61	0.65	0.58	0.55	0.48	0.58
9								1.00	0.73	0.77	0.69	0.67	0.55	0.70
10									1.00	0.75	0.67	0.64	0.47	0.68
11										1.00	0.76	0.68	0.67	0.71
12											1.00	0.78	0.70	0.81
13												1.00	0.75	0.88
14													1.00	0.72

and genetic markers (RAPD). The use of RAPD markers has been of great importance in the characterization and identification of genetic variation and relationships between different genotypes and radiation treatments with high accuracy. The developed mutant materials will be subjected to distinct, uniformity and stability trials for resistance to abiotic stress, in particularly drought and salinity for development of new grapevine rootstocks adapted for Egyptian condition.

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التنوع الوراثي والعلاقات بين طفرات أصول العنب باستخدام تكنيك RAPD

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استخدام الاشعاع في احداث طفرات يعتبر وسيله مهمه في دمج صفات وراثية ذات قيمة نوعية ومرغوبة. في هذه الدراسة استخدم أربع أصول من العنب (فريدم – هرموني –SO4 – رامسي) مع صنف تجارى وهو العنب البناتي، حيث تعرضت عقل من هذه الأصول لاشعة جاما بجرعات مختلفة (صفر – 10 – 20 جرى) وذلك بغرض الحصول على طفرات ذات قيمة اقتصادية مرغوبة لتراكيب العنب الوراثية.

واظهرت النتائج تغير في النسبة المئوية لتفتح البراعم، وطول الافرع وعدد الأوراق. وتم تقدير العلاقات الوراثية بين أصول العنب الأربعة باستخدام تقنية التضخيم العشوائي للحمض النووي RAPD باستخدام تفاعل PCR. واتضح من اختبار RAPD اختلاف وراثي بين العقل المعاملة بالإشعاع والغير معامله وتم تقسيم الجينوتايب الى مجموعتين حيث وجد ان أصل العنب فريدم والمعامل ب 10 جرى يقع في مجموعة والتسعة الأخرى المعاملة بالإشعاع تقع في المجموعة الثانية ويتراوح التشابه الجيني من 0.61 الى 0.93. وأوضحت النتائج ان أصول العنب المستخدمة تعد ماده وراثية واعدة يمكن استخدامها في برامج تربيه وتحسين العنب لإنتاج أصناف ذات صفات وراثية مرغوبة.

التوصية: تدعم هذه الدراسة استخدام الطفرات في تربية وتحسين كروم العنب، كما أن استخدام تقنية RAPDيمكن أن تكون أداة مهمة للإستدلال على حدوث الطفرات وكذلك الاستدلال على التنوع الوراثي خلال التراكيب الوراثية لكروم العنب.