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ISSR Markers Associated With Effects of Gamma Irradiation on Growth and Seed Yield of M2 Plants of Faba Bean (Vicia faba L.)

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Abstract

Dry seeds of six faba bean varieties (Sakha 1, Nubaria 2, Nubaria 3, Giza 3, Giza 716 and Giza 843) were irradiated with low doses of gamma rays (20, 40, 60, 80, 100 and 200 Gy). Treated seeds and control seeds were grown for two seasons. Results revealed that doses below 100 Gy increased morphological growth parameters as well as some yield parameters. The most effective dose varied in different faba bean varieties. Higher doses above 100 Gy were detrimental to plant growth and yield. The variation in ISSR profiling in response to g-irradiation treatments in M2 was detected using seven ISSR primers. The seven primers produced 81 bands including 75 polymorphic bands and 6 unique bands. The unique bands were scored in var. Nubaria 3 by the doses (20, 80 and 100 Gy), var. Giza 3 by the doses (20 and 60 Gy) and var. Nubaria 2 by the dose 200 Gy. The two varieties Nubaria 3 and Nubaria 2 showed higher numbers of polymorphic bands (225, 191) respectively compared to other varieties. Polymorphic information content (PIC) was estimated for each primer and ranged from 0.193 for primer 807 to 0.31 for primer 834 with a mean of 0.259.

Keywords*: Mutation breeding, Gamma rays, Faba bean, Growth, Yield, ISSR.*

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Introduction

Faba bean or broad beans (*Vicia faba* L.; 2n = 12) is a major food and feed grain legume owing to the high nutritional value of its seeds (Link *et al*., 1995; Duc, 1997). It is considered as one of the major sources of cheap protein in Africa, parts of Asia and Latin America as reported by Duc (1997) and Alghamdi (2009). It is a dual-purpose crop, which not only provides inexpensive proteins for human consumption (particularly in western Asia and northern Africa), but also serves as a prime livestock feed in Europe and Australia (Alghamdi *et al*., 2012; Ellwood *et al*., 2008; Torres *et al*., 2010). In Egypt, faba bean is among the main nutritional source of plant proteins (El-Danasoury *et al*., 2008; Bakry *et al*., 2011). Nevertheless, the total production of this crop falls short of covering the increasing consumption, so there is a need to increase the production by expanding faba bean cultivation in reclaimed lands in different parts of the country (Khalafallah *et al*., 2008; Bakry *et al*., 2011).

Mutation breeding has been used in plant improvement programs to cause genetic variability that enables plant breeders to select new genotypes with improved characteristics. In many cases, radiation induced mutants that were developed into released mutant varieties of desired characters in breeding programs to produce better crop plants (Maluszynski *et al*., 2000). Gamma rays represent one of the important physical agents used to improve the characters and productivity of many plants as low doses of g-irradiation have been used for mutant isolation in conventional plant breeding (Jaywardena and Peiris, 1988; Chopra, 2005).

However, high doses can be detrimental reducing germination, growth rate, vigor and pollen and ovule fertility and yield (Singh, 2005; Badr *et al.*, 2014).

Recently, DNA markers have become gradually more utilized for routine testing of the genetic diversity (Gilbert *et al*., 1999; Badr and El-Shazly, 2012). The most commonly used methods for genetic diversity and relationships in faba bean have been the randomly amplified polymorphic DNA (RAPD) (Link *et al*., 1995; El-Danasoury *et al*., 2008), amplified fragment length polymorphism (AFLP) (Zeid *et al*., 2003; Duc *et al*., 2010) and simple sequence repeats (Zeid *et al*., 2009). The main limitations of these methods are low reproducibility of RAPD, high cost of AFLP and the necessity to know the flanking sequences to develop species specific primers for SSR polymorphism (Belaj *et al*., 2003). Inter-simple sequence repeats (ISSRs) have been developed to overcome most of these limitations. The ISSR markers provide a quick, reliable and highly informative system for DNA fingerprinting to generate species-specific genomics fingerprints (Zietkiewicz *et al*., 1994; Reddy *et al*., 2002). It is a fast, simple, cost-efficient and does not require any prior knowledge about the sequences to be amplified. ISSR analysis has been successfully applied to determine genetic diversity and relationships in numerous economic legume species such as cowpea (Ajebade *et al*., 2000; Badr *et al*., 2014), common bean (Galvan *et al*., 2003; Gonzales *et al*., 2005), chickpea (Sudupak, 2004), in addition to faba bean (Terzopoulosa and Bebeli, 2008).

The aim of this work was to investigate the response of six faba bean varieties to different doses of gamma irradiation (20, 40, 60, 80, 100 and 200 Gy) in terms of vegetative growth and seed yield. The ISSR markers have been investigated in the six varieties following exposure flowing exposure to gamma radiation doses.

Materials and methods

Plant material and Seeds irradiation and production of M2 plants

Seeds of six faba bean (*Vicia faba* L.) varieties (Sakha 1, Nubaria 2, Nubaria 3, Giza 3, Giza 716, and Giza 843) were obtained from the Legume Crops Research Department, Field Crops Research Institute (FCRI), Agricultural Research Center (ARC), Giza, Egypt. The dry seeds of the faba bean varieties were irradiated with the following doses of gamma rays: 20, 40, 60, 80, 100 and 200 Gy at the National Center for Radiation Research and Technology (NCRRT), Egyptian Atomic Energy Authority (EAEA), Nasr city, Cairo, Egypt using cobalt 60 as a source. Seeds of control samples were not exposed to irradiation. Treated and untreated seeds of the six faba bean varieties were grown to maturity in the Botanical Garden of the Botany Department, Faculty of Science, Suez Canal University in Ismailia, Egypt from November 2013 to April 2014. Seeds were collected from all M1 plants (first generation plants) except for the plants of the varieties Giza 716 and Giza 843 following exposure 200 Gy which did not produce seeds in the first generation. The seeds of the M1 generation were then sown and grown in the field from November 2014 to April 2015 for the present study.

Morphological measurements

Vegetative growth characters and Seed yield

After six weeks from sowing, the dry weight of shoot and root was recorded for M2 using three random plants from each treatment which dried in an oven at 70°C for 48 h. After 12 weeks from sowing, the following growth criteria were recorded, using three random plants for each treatment; shoot length, root length, shoot fresh weight and root fresh weight. At maturity and full ripening the productivity of the M2 plants (second generation plants) was measured as number of pods per plant, pod length and number of seeds per pod. In addition the weight of 100 seed was determined at the end of the flowering stage (after 24 weeks from sowing.

Statistical analysis

The comparison of means was done with 'One Way Analysis of Variance' (ANOVA) using SPSS V19 software at probability level of 0.05%.

ISSR analysis

Total genomic DNA extraction

Total genomic DNA was extracted according to the basic DNA extraction protocol of Dellaporta *et al*. (1983) with slight modifications by Porebski *et al*. (1997) and adapted to faba bean for obtaining good quality. In brief, 200 mg young leaves were ground in liquid nitrogen to fine powder and extracted using 1 ml preheated (65ºC) cetylhexadecyl-trimethyl ammonium bromide (CTAB) extraction buffer (40 ml CTAB, 2g PVP (Polyvinyl pyrrolidone). The CTAB/plant extract mixture was incubated at 65ºC for 60 min, then the tubes incubated on ice for 5 mins, followed by adding 1 ml chloroform/isoamyl alcohol (24:1), the tubes were inverted slowly many times to precipitate the DNA for 20 mins then centrifuged at 12000 rpm at 4ºC for 15 mins. The supernatant (containing the DNA) was transferred to another tube and 1 ml of cold isopropanol was added and the solution mixed by inversion for few seconds. Then the tubes were incubated on ice for 30-40 minutes. The tubes were centrifuged at 12000 rpm at 4ºC for 5 mins. The supernatant was carefully removed and 1 ml of chilled ethanol (70%) was added to the pellet, and then centrifuged at 12000 rpm at 4ºC for 5 mins. The DNA was washed twice with 70% chilled ethanol, then the supernatant was removed and the DNA pellet allowed to dry for 15 min and then was dissolved in 50 µl Tris EDTA buffer. The purified total DNA was quantified by gel electrophoresis, DNA samples were then stored in the freezer until use for ISSR fingerprinting.

PCR (ISSR) amplification and product electrophoresis

Seven random primers (Sigma, Germany) were selected for ISSR analysis (Table 1). The ISSR amplification was carried out in a thermocycler (Eppendorf Master Cycler Gradient, Hamburg, Germany). PCR reactions were performed in 20 µl mixture containing 1µl of template DNA, 2 µl of dNTPs, 2 µl of primer, 2 µl $Mgcl_2$, 4 µl buffer, 0.3 µl Taq DNA polymerase and 8.7 µl PCR water. After a first denaturation step at 94ºC for 7 min., the reaction went through 45 cycles with denaturing step at 94ºC for 30 sec., an annealing step at 52ºC for 45 sec., an extension step at 72ºC for 2 min. and the last cycle was closed by 72ºC for 5 min. Amplified products were fractionated by electrophoresis in 1.5% agarose gel containing ethidium bromide. The gels were photographed with ULTRA-LUM equipment.

Table 1: List of ISSR primers used in the amplification reaction for ISSR finger-printing in the examined six varieties of faba bean following exposure to different doses of g-radiation showing the number of bands, unique bands, PIC and PIC per band for each primer

Primer No.	Primer code	Primer sequence (5'—3')	No of bands	Unique bands	PIC	PIC per band
1	857	ACA CAC ACA CAC ACA CYG	12		2.973	0.247
2	841	GAG AGA GAG AGA GAG AYC	8	1	2.433	0.304
3	835	AGA GAG AGA GAG AGA GYC	10		2.740	0.274
4	834	AGA GAG AGA GAG AGA GYT	14	1	2.610	0.261
5	810	GAG AGA GAG AGA GAG AT	10	1	2.127	0.193
6	807	AGA GAG AGA GAG AGA GT	11	1	4.289	0.306
7	HB12	CAC CAC CAC GC	16	2	3.806	0.237
Total			81	6		

Results

Effect of irradiation on vegetative growth and yield

The values of the measured vegetative and yield traits in the M2 plants of all varieties of faba bean, following exposure to the applied g-radiation doses, are given in Table 2. The seeds obtained from M1 plants of the two varieties Giza 716 and Giza 843 following exposure to the dose 200 Gy did not germinate to give M2 plants. In general, doses up to 60 Gy resulted in increases in the measured morphological and yield traits of the three varieties Sakha 1, Nubaria 2 and Nubaria 3 and Giza 716. The highest increase in the examined traits was induced by the dose of 60 Gy in Sakha 1 and Nubaria 2 and by the dose of 40 Gy in the two varieties Nubaria 3. On the other hand doses of 40 Gy 20 Gy and resulted in increases in the measured vegetative and yield traits of var. Giza 2 and Giza 716 respectively. Contrary to the above increases in the vegetative and yield traits by low doses of g radiation, all doses resulted in reduction in the measured traits in var. Giza 843.

Table 2: Effect of different doses of gamma irradiation (20, 40, 60, 80, 100 and 200 Gy) on growth parameters and seed yield traits of M2 plants of the six faba bean cultivars Sakha1, Nubaria 2, Nubaria 3, Giza 3, Giza 716 and Giza 843.

Variety	Shoot length (cm)	Root length (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)	Pod length (cm)	No. of pods/plant	No. of seeds/pod	100-seed weight (g)
Sakha1 0	63.00 ± 0.40	14.50 ± 0.46	79.80 ± 0.49	12.40 ± 0.31	1.65 ± 0.03	0.75 ± 0.03	8.50 ± 0.25	8.00 ± 0.58	3.00 ± 0.58	92.55 ± 0.54
20	74.20 [a] ± 0.42	15.25 ± 0.16	82.40 [a] ± 0.31	14.20 [a] ± 0.12	1.75 ± 0.08	0.95 [a] ± 0.08	10.00 [a] ± 0.29	9.00 ± 0.58	3.33 ± 0.33	95.30 [a] ± 0.30
40	79.30 [a] ± 0.26	16.90 [a] ± 0.15	85.20 [a] ± 0.36	15.15 [a] ± 0.29	2.15 [a] ± 0.05	1.05 [a] ± 0.05	12.00 [a] ± 0.25	10.50 [a] ± 0.29	4.00 ± 0.58	106.50 [a] ± 0.61
60	93.20 [a] ± 0.53	18.30 [a] ± 0.26	89.40 [a] ± 0.31	17.30 [a] ± 0.40	2.45 [a] ± 0.05	1.15 [a] ± 0.03	10.00 [a] ± 0.17	13.00 [a] ±0.58	3.00 ± 0.00	94.55 [a] ± 0.74
80	71.30 [a] ± 0.57	14.90 [a] ± 0.23	81.30 [a] ± 0.67	13.20 ± 0.26	1.69 ± 0.01	0.83 ± 0.07	9.70 [a] ± 0.21	11.00 [a] ±0.50	3.00 ± 0.58	96.60 [a] ± 0.60
100	48.0 [a] ± 0.47	10.23 [a] ±0.32	28.40 [a] ±0.32	6.70 [a] ± 0.21	1.15 [a] ± 0.08	0.43 [a] ± 0.09	8.20 ± 0.12	8.50 ± 0.76	3.33 ± 0.33	71.05 [a] ±0.53
200	46.20 [a] ± 0.51	8.70 [a] ± 0.31	26.30 [a] ±0.32	5.30 [a] ± 0.23	0.53 [a] ± 0.03	0.19 [a] ± 0.01	8.00 ± 0.23	7.00 ± 0.17	2.00 ± 0.00	60.40 [a] ±0.31
Nubaria2 0	69.00 ± 0.26	9.50 ± 0.10	50.30 ± 0.30	6.15 ± 0.15	1.45 ± 0.05	0.52 ± 0.02	7.50 ± 0.29	15.00 ± 0.29	2.33 ± 0.33	77.50 ±0.26
20	73.50 [a] ± 0.64	10.45 [a] ± 0.10	54.20 [a] ± 0.61	6.75 ± 0.32	1.83 [a] ± 0.07	0.54 ± 0.03	8.50 [a] ± 0.25	17.50 [a] ±0.36	3.00 ± 0.00	102.00 [a] ±0.53
40	75.50 [a] ± 0.49	11.93 [a] ± 0.15	56.40 [a] ± 0.40	6.85 ± 0.22	1.93 [a] ± 0.03	0.56 ± 0.04	9.50 [a] ± 0.26	18.50 [a] ± 0.40	3.00 ± 0.58	105.00 [a] ±0.58
60	78.50 [a] ± 0.72	12.25 [a] ± 0.16	75.15 [a] ± 0.31	9.35 [a] ± 0.09	2.55 [a] ± 0.05	0.62 [a] ± 0.01	10.70 [a] ± 0.21	25.00 [a] ±0.50	3.00 ±0.00	111.64 [a] ±0.95
80	70.40 ± 0.67	9.75 ± 0.14	39.50 [a] ± 0.36	5.80 ± 0.23	1.50 ± 0.06	0.53 ± 0.01	9.75 [a] ±0.25	16.00 ± 0.58	2.33 ± 0.33	103.30 [a] ±0.78
100	57.30 [a] ± 0.44	8.80 [a] ± 0.31	32.70 [a] ± 0.15	4.40 [a] ± 0.31	1.13 [a] ± 0.09	0.48 ± 0.04	10.00 [a] ± 0.25	8.00 [a] ± 0.29	4.00 [a] ± 0.58	94.30 [a] ±0.85
200	50.20 [a] ± 0.53	8.50 [a] ± 0.26	30.30 [a] ± 0.21	4.35 [a] ± 0.24	0.95 [a] ± 0.03	0.46 ± 0.02	7.50 ± 0.26	6.50 [a] ±0.58	3.33 ± 0.33	78.88 ± 0.44
Nubaria3 0	82.50 ± 0.32	12.90 ± 0.47	89.30 ± 0.40	10.30 ± 0.30	1.68 ± 0.02	0.47 ± 0.02	8.40 ± 0.12	11.00 ± 0.29	2.00 ± 0.00	91.20 ±0.20
20	93.20 [a] ± 0.29	13.23 ± 0.27	92.70 [a] ± 0.29	11.15 [a] ± 0.18	1.84 ± 0.06	0.59 ± 0.07	8.50 ± 0.17	16.00 [a] ± 0.58	3.00 ± 0.58	95.90 [a] ±0.45
40	95.20 [a] ± 0.61	13.50 ± 0.38	103.30 [a] ± 0.21	12.25 [a] ± 0.14	2.35 [a] ± 0.05	0.70 [a] ± 0.06	10.50 [a] ± 0.23	20.00 [a] ± 0.29	3.00 ± 0.00	101.80 [a] ± 0.96
60	85.30 [a] ± 0.40	13.17 ± 0.15	90.67 [a] ± 0.46	10.39 ± 0.22	1.79 ± 0.05	0.54 ± 0.03	11.00 [a] ± 0.17	15.00 [a] ± 0.50	3.00 ± 0.00	114.65 [a] ±0.66
80	74.20 [a] ± 0.42	11.20 [a] ± 0.38	79.80 [a] ± 0.38	10.03 ± 0.09	1.53 ± 0.09	0.41 ± 0.05	9.80 [a] ±0.12	12.00 ± 0.29	4.00 [a] ± 0.58	105.70 [a] ±0.70
100	70.00 [a] ± 0.42	9.43 [a] ± 0.15	69.30 [a] ± 0.35	9.85 ± 0.28	1.23 [a] ± 0.03	0.32 [a] ± 0.02	8.70 ± 0.15	14.00 [a] ±0.29	3.00 ± 0.00	97.65 [a] ± 0.33
200	67.30 [a] ± 0.68	9.00 [a] ± 0.40	46.17 [a] ± 0.20	8.20 [a] ± 0.12	1.07 [a] ± 0.07	0.30 [a] ± 0.01	8.00 ± 0.17	8.00 [a] ± 0.58	2.33 ± 0.33	91.75 ±0.63
Giza3 0	69.50 ± 0.67	10.80 ± 0.53	70.30 ± 0.52	8.70 ± 0.38	1.56 ± 0.07	0.51 ± 0.01	9.00 ± 0.23	10.00 ± 0.17	3.33 ± 0.33	82.80 ± 0.75
20	71.25 ± 0.38	11.10 ± 0.31	71.20 ± 0.21	8.90 ± 0.35	1.81 [a] ± 0.07	0.54 ± 0.01	10.00 [a] ± 0.17	16.67 [a] ± 0.24	3.33 ± 0.33	90.00 [a] ± 0.50
40	76.60 [a] ± 0.67	11.30 ± 0.38	78.40 [a] ± 0.55	11.70 [a] ± 0.52	2.05 [a] ± 0.05	0.68 [a] ± 0.02	9.75 [a] ± 0.26	23.60 [a] ±0.60	3.00 ± 0.00	99.00 [a] ± 0.29
60	64.00 [a] ± 0.76	9.20 [a] ± 0.12	58.30 [a] ± 0.61	7.70 ± 0.31	1.42 ± 0.08	0.47 ± 0.02	9.50 ± 0.29	15.00 [a] ± 0.46	4.00 ± 0.00	99.28 [a] ± 0.36
80	60.50 [a] ± 0.74	8.80 [a] ± 0.15	33.70 [a] ± 0.36	7.20 [a] ± 0.20	1.21 [a] ± 0.01	0.44 [a] ± 0.03	8.70 ± 0.15	11.00 ± 0.50	3.00 ± 0.58	90.17 [a] ± 0.60
100	49.00 [a] ± 0.29	8.30 [a] ± 0.30	23.40 [a] ± 0.42	4.15 [a] ± 0.08	1.13 [a] ± 0.09	0.31 [a] ± 0.01	7.60 [a] ± 0.21	9.00 ± 0.29	2.67 ± 0.33	88.53 [a] ± 0.55
200	46.40 [a] ± 0.64	8.07 [a] ± 0.38	21.50 [a] ± 0.66	4.05 [a] ± 0.37	1.05 [a] ± 0.05	0.28 [a] ± 0.02	6.50 [a] ± 0.25	8.00 [a] ± 0.17	2.33 ± 0.33	64.55 [a] ± 0.75
Giza716 0	60.00 ± 0.71	9.55 ± 0.13	69.20 ± 0.59	10.25 ± 0.38	1.21 ± 0.01	0.45 ± 0.03	9.25 ± 0.14	13.00 ± 0.23	3.00 ± 0.58	102.44 ± 0.80
20	80.50 [a] ± 0.36	15.30 [a] ± 0.25	88.60 [a] ± 0.31	21.33 [a] ± 0.33	1.78 [a] ± 0.04	0.82 [a] ± 0.02	10.00 [a] ±0.17	21.00 [a] ± 0.76	2.67 ± 0.33	108.17 [a] ±0.27
40	70.30 [a] ± 0.38	13.20 [a] ± 0.15	87.30 [a] ± 0.30	15.30 [a] ± 0.30	1.65 [a] ± 0.08	0.51 ± 0.02	9.75 ± 0.25	12.00 ± 0.29	3.00 ± 0.00	113.20 [a] ± 0.42
60	67.50 [a] ± 0.36	12.30 [a] ± 0.17	86.40 [a] ± 0.31	14.20 [a] ± 0.12	1.56 [a] ± 0.05	0.48 ± 0.03	9.00 ± 0.23	11.00 [a] ± 0.50	2.67 ± 0.33	113.10 [a] ±0.38
80	57.40 [a] ± 0.26	9.00 ± 0.36	62.60 [a] ± 0.96	9.20 [a] ± 0.20	1.24 ± 0.04	0.32 [a] ± 0.02	8.50 [a] ± 0.29	10.00 [a] ±0.25	3.00 ± 0.00	103.12 [a] ± 0.48
100	50.20 [a] ± 0.42	8.50 [a] ± 0.29	61.30 [a] ± 0.70	8.90 [a] ± 0.10	0.85 [a] ± 0.08	0.27 [a] ± 0.02	5.70 [a] ± 0.21	8.00 [a] ± 0.25	1.67 [a] ± 0.33	98.12 [a] ± 0.68
Giza834 0	65.30 ± 0.61	13.50 ± 0.36	60.30 ± 0.59	9.05 ± 0.05	2.25 ± 0.03	0.58 ± 0.01	6.75 ± 0.25	10.00 ± 0.50	3.00 ± 0.58	98.60 ±0.38
20	61.20 [a] ± 0.67	13.37 ± 0.47	45.53 [a] ± 0.35	8.10 [a] ± 0.10	1.75 [a] ± 0.03	0.50 [a] ± 0.02	7.00 ± 0.12	15.00 [a] ± 0.29	2.00 [a] ±0.00	94.84 [a] ± 0.60
40	57.20 [a] ±0.61	12.40 ± 0.31	42.30 [a] ± 0.64	6.30 [a] ± 0.17	1.62 [a] ± 0.04	0.42 [a] ± 0.02	7.00 ± 0.17	13.00 [a] ± 0.50	2.00 [a] ± 0.00	97.17 ± 0.60
60	55.40 [a] ± 0.76	12.00 [a] ± 0.76	38.50 [a] ± 0.26	6.03 [a] ± 0.15	1.13 [a] ± 0.09	0.40 [a] ± 0.01	10.00 [a] ± 0.25	10.00 ± 0.29	3.00 ± 0.00	87.00 [a] ±0.58
80	50.30 [a] ± 0.32	9.00 [a] ± 0.29	25.33 [a] ± 0.33	4.20 [a] ± 0.12	1.03 [a] ± 0.03	0.19 [a] ± 0.03	7.50 [a] ± 0.25	9.00 ± 0.25	2.67 ± 0.33	87.57 [a] ± 0.43
100	41.00 [a] ± 0.26	8.00 [a] ± 0.40	12.30 [a] ± 0.42	3.70 [a] ± 0.21	0.63 [a] ± 0.09	0.15 [a] ± 0.03	7.00 ± 0.23	8.00 [a] ± 0.35	3.00 ± 0.00	71.08 [a] ± 0.46

0 refers to the control, the data applied in the form mean ±SE, [a] treatment significant versus untreated control using LSD's post-hoc statistical analysis test at p-value<0.05.

The maximum shoot length (95.20±0.61cm) was scored in var. Nubaria 3 by the dose 40 Gy. Meanwhile the shortest shoot length was 39.5±0.31 cm and scored in var. Giza 843 by the dose 100 Gy. The maximum root length (18.30±0.26 cm) was scored in var. Sakha 1 by the dose 60 Gy. Meanwhile the shortest root length (8.00±0.40 cm) was scored in Giza 843 by the dose 100 Gy. The maximum shoot fresh weight was 103.30±0.21 g and scored in var. Nubaria 3 by the dose 40 Gy. Meanwhile the lowest shoot fresh weight was 12.30±0.40 g and scored in Giza 843 by the dose 100 Gy.

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The maximum root fresh weight was 21.33 ±0.33 g and scored in var. Giza 716 by the dose 20 Gy compared to 10.25 ±0.38 g for the control. Meanwhile, the lowest root fresh weight was 3.70±0.21 g and scored in var. Giza 843 by the dose 100 Gy. The maximum shoot dry weight was 2.55±0.05 g scored in var. Nubaria 2 by the dose 60 Gy while the lowest shoot dry weight (0.53±0.03 g) was scored in var. Sakha 1 by the dose 200 Gy. The maximum root dry weight was 1.15 ±0.03 g and scored in var. Sakha 1 by the dose 60 Gy. Meanwhile, the lowest root dry weight was 0.15±0.03 g scored in Giza 843 by the dose 100 Gy.

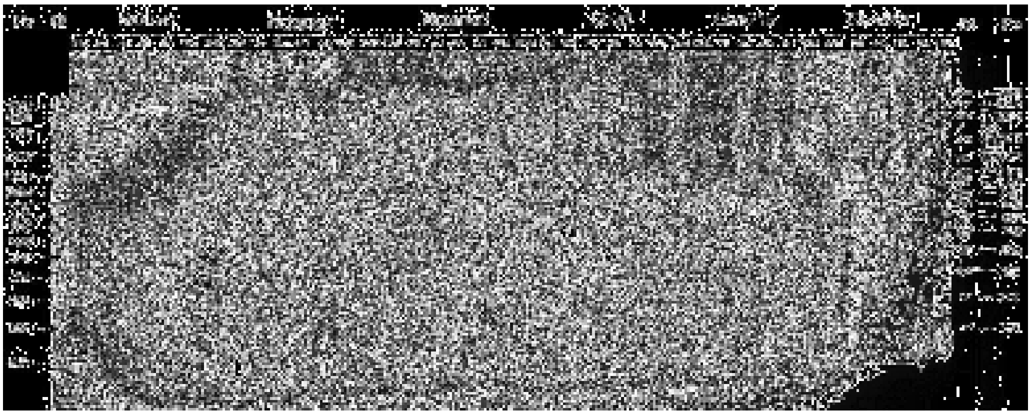
The data given in Table 2 also show that the maximum number of pods per plant was 25.00±0.50 and was produced by the var. Nubaria 2 by the dose 60 Gy, while the lowest number of pods per plant was 6.50±0.58 and was also scored in the var. Nubaria 2 by the dose 200 Gy of g-radiation illustrating the detrimental action of the high doses of g-radiation. The maximum pod length was 12.00±0.25 cm in var. Sakha 1 by the dose of 40 Gy while the lowest pod length was 6.50±0.25 cm in var. Giza 3 by the dose of 200 Gy. The highest number of seeds per pod was 4.00±0.58 in var. Sakha 1 by the dose of 40 Gy and also in var. Giza 3 by the dose of 60 Gy and in var. Nubaria 3 by 80 Gy while the lowest number of seeds per pod was 1.67 ± 0.33 in var. Giza 716 by the dose of 200 Gy. The maximum 100-seed weight was 114.65±0.66 g in var. Nubaria 3 by the dose of 60 Gy, while the lowest 100-seed weight was 60.40±0.31 g in var. Sakha 1 by the dose of 200 Gy.

ISSR-PCR of genomic DNA in M2 plants

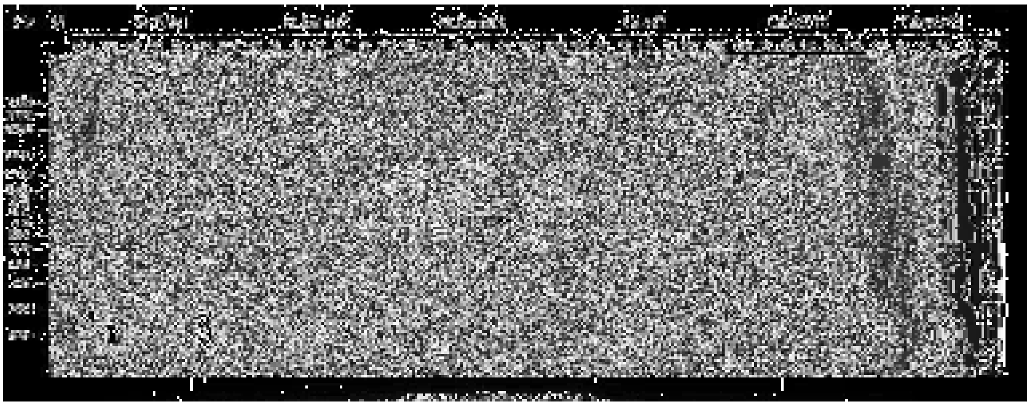
The ISSR bands were scored as 1 for presence and 0 for absence of markers for estimating genetic variation as a result of g-radiation. The seven ISSR primers amplified 81 bands, 75 bands were polymorphic and six bands were unique bands. The ISSR profiles for the M2 genotypes are illustrated in Figure 1. The percentage of polymorphism of the amplified products was 100%. The bands ranged in size between 150 pb and 2000 pb. The number of bands, unique bands as well as the PIC for the seven primers and the PIC per band in the examined varieties are given in Table (1).

Primer 857 revealed 12 bands ranging in size between 230 to 1350 pb; all of them were polymorphic. The highest number of bands (9) was found in var. Nubaria 3 by the dose 100 Gy of g-radiation. Polymorphic information content (PIC) for this primer was 2.973 with a mean of 0.247 for each band.

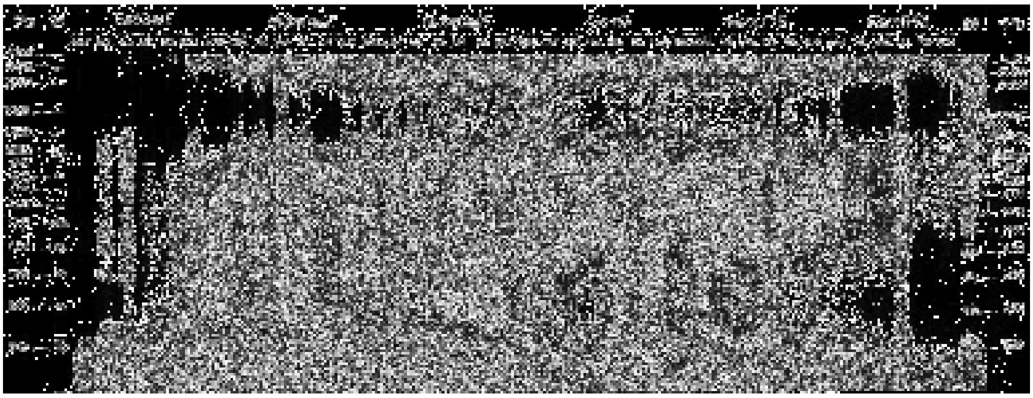
Primer 841 revealed 8 bands ranging in size between 170 to 900 pb; seven bands were polymorphic and one band was unique with molecular size 700 pb in var. Nubaria 3 by the dose 100 Gy. The highest number of bands (6) was scored in var. Sakha 1 by the dose 60 Gy of g-radiation and in var. Nubaria 3 by the dose of 100 Gy and the control plants of Giza 716. The PIC for this primer was 2.433 with a mean of 0.304 for each band. The primer 835 revealed 10 bands ranging in size between 190 to 1100 pb, all of them are polymorphic. The highest numbers of bands (7 and 8) were scored in var. Nubaria 3 by the doses of 100 and 200 Gy respectively. The PIC for this primer was 2.740 with a mean of 0.274 for each band. Primer 834 revealed 14 bands ranging in size between 150 to 2000 pb; 13 bands were polymorphic and one band was unique with molecular size 1500 pb in Giza 3 by the dose 20 Gy. The highest number of bands (11) was scored in var. Nubaria 3 by the dose 60 Gy. The PIC for this primer was 2.610 with a mean of 0.261 for each band.



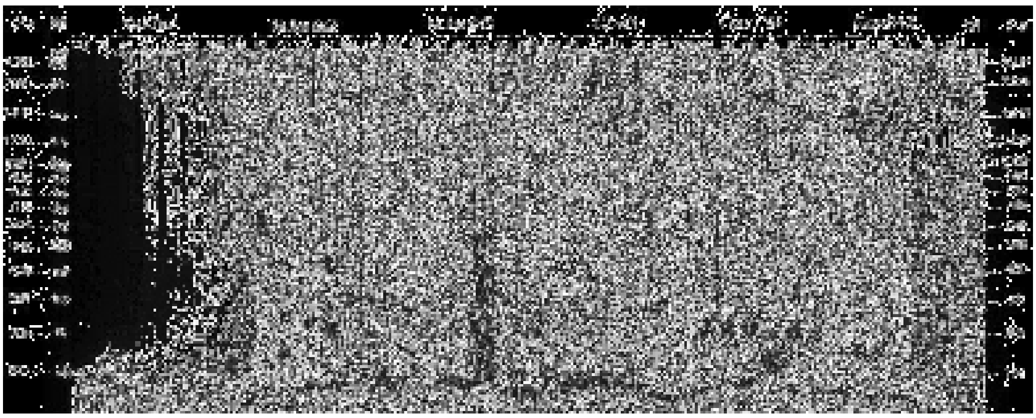
Primer857



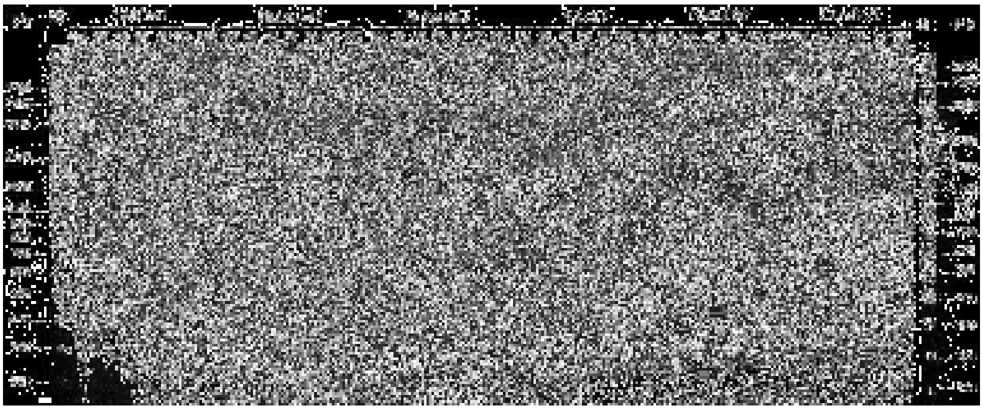
Primer 841



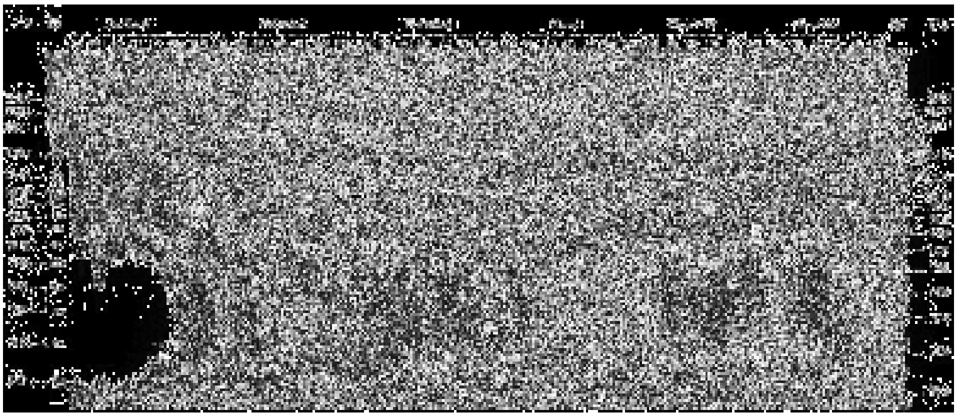
Primer835



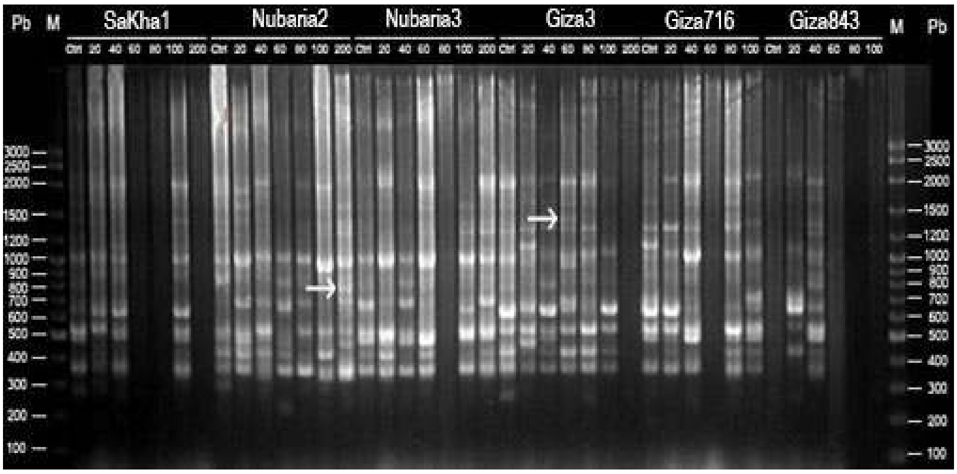
Primer 834



Primer 810



Primer 807



Primer HB12

Figure 1: ISSR profiles produced in M2 plants of the six faba bean varieties following seed exposure to different doses of gamma radiation by seven primers.

Primer 810 revealed ten bands ranging in size between 200 to 1250 pb; 9 bands were polymorphic and 1 band was unique with molecular size 1000 pb in var. Nubaria 3 by the dose 80 Gy. The highest number of bands (7) was scored in var. Nubaria 2, in the control plants and by the dose of 60 Gy), in var. Nubaria 3 by the dose of 80 Gy and in var. Giza 3 by the dose 60 Gy. The PIC for this primer was 2.127 with a mean of 0.193 for each band.

Primer 807 revealed 11 bands ranging in size between 250 to 1350 pb; ten bands were polymorphic and one band was unique with molecular size 1000 pb in var. Nubaria 3 by the dose 20 Gy. The highest number of bands (6) was scored in var. Nubaria 3 by the dose of 200 Gy and in var. Sakha 1 by the dose of 100 Gy. The PIC for this primer was 4.289 with a mean of 0.306 for each band.

Primer HB12 revealed 16 bands ranging in size between 200 to 2000 pb, 14 bands were polymorphic and two bands were unique with molecular size 800 pb in var. Nubaria 2 by the dose 200 Gy and 1400 pb in var. Giza 3 by the dose 60 Gy. The highest number of bands (10) was scored in var. Giza 843 by the dose 40 Gy. The PIC for this primer was 3.806 with a mean of 0.237 for each band.

The number of bands in each treatment with each primer in the M2 plants of the six faba bean varieties after seeds exposure to some doses of gamma radiation (20, 40, 60, 80, 100 and 200 Gy) was illustrated in table (4) and the total number of bands found in all treatments for each primer and the total number of bands observed in each variety was shown in table (3).The data in this table showed that the primers which produce more bands than the other primers were 834 (202 bands) and HB12 (191 bands) whereas the least number of bands was (60) which given by primer 807. The number of bands in each variety as illustrated in Table (3) was (225) in var. Nubaria 3, (191) in var. Nubaria 2, (181) in var. Giza 3, (145) in var. Giza 716, (80) in var. Sakha 1 and (39) in var. Giza 843.

The highest number of bands in the treatments of var. Sakha 1 was (21) by the dose of 100 Gy, (36) in var. Nubaria by the dose of 60 Gy, (47) in var. Nubaria 3 by the dose of 100 Gy, (37) in var. Giza 716 by the dose of 80 Gy and (19) in var. Giza 843 by the dose of 40 Gy while in var. Giza 716 all the treatments gave number of bands less than the control. Finally, the primer which gave the highest number of bands (202) was primer 834, the variety which gave the highest number of bands (225) was var. Nubaria 3 and the treatment which gave the highest number of bands (47) was 100 Gy in var. Nubaria 3 as revealed in table (4).

Table (3): The total number of bands found in all treatments for each primer and the total number of bands observed in each variety.

Variety / Primer name	Sakha1	Nubaria2	Nubaria 3	Giza3	Giza716	Giza843	Total no. of bands
Primer857	0	12	30	29	18	6	95
Primer841	16	14	29	19	26	6	109
Primer835	17	30	35	12	8	0	102
Primer834	1	42	51	46	50	12	202
Primer810	19	28	25	24	5	1	102
Primer807	6	23	20	8	4	0	60
PrimerHB12	21	42	35	44	34	15	191
Total no. of bands	80	191	225	181	145	39	861

Table (4): Number of bands in each treatment with each primer in the M2 plants of six faba bean varieties after seeds exposure to some doses of gamma radiation (0, 20, 40, 60, 80, 100 and 200 Gy).

Primer name	Variety and dose of g- radiation																																								Total no. of bands
	Sakha1							Nubaria 2							Nubaria 3							Giza 3							Giza 716						Giza 843						
	0	20	40	60	80	100	200	0	20	40	60	80	100	200	0	20	40	60	80	100	200	0	20	40	60	80	100	200	0	20	40	60	80	100	0	20	40	60	80	100	
857	0	0	0	0	0	0	0	0	4	4	4	0	0	0	3	2	0	4	8	9	4	0	7	5	5	5	1	6	0	5	5	2	6	0	6	0	0	0	0	0	95
841	5	1	0	6	0	4	0	0	0	0	5	0	5	4	3	3	5	3	4	6	5	2	4	0	4	4	5	0	6	5	3	3	4	5	0	3	2	0	0	0	109
835	0	6	6	0	0	5	0	3	5	5	8	0	4	5	6	6	4	5	0	7	7	7	0	0	4	1	0	0	0	0	4	0	4	0	0	0	0	0	0	0	102
834	0	0	0	0	0	0	1	6	6	6	3	5	8	8	7	6	8	11	0	9	10	8	9	8	8	8	5	0	8	8	10	6	10	8	0	5	7	0	0	0	202
810	2	4	3	4	6	0	0	7	3	3	7	4	4	0	3	4	1	5	7	5	0	6	6	7	5	0	0	0	0	0	0	0	3	2	0	0	0	0	0	1	102
807	0	0	0	0	0	6	0	4	2	4	4	0	5	4	1	5	0	4	0	4	6	4	0	0	1	2	0	0	2	0	0	0	2	0	0	0	0	0	0	0	60
HB12	5	5	5	0	0	6	0	6	7	5	5	6	5	8	5	5	5	7	0	7	6	8	8	7	8	8	5	0	7	6	6	0	8	7	0	5	10	0	0	0	191
Total no. of bands	12	16	14	10	6	21	1	26	27	27	36	15	31	29	28	31	23	39	19	47	38	35	34	27	35	28	16	6	23	24	28	11	37	22	6	13	19	0	0	1	861

DISCUSSION

The foregoing results revealed that low doses of g-radiation induced an increase in the vegetative growth parameters and yield components of faba bean. The magnitude of increase varied between varieties and the dose used. Varieties Sakha 1 and Nubaria 2 have higher shoot and root length, shoot and root fresh weight as well as shoot and root dry weight compared to the control following seed exposure to 60 Gy. The varieties Nubaria 3 and Giza 3 showed higher values of the same parameters at 40 Gy while var. Giza 716 showed higher values at 20 Gy. Some doses of g-radiation enhanced the yield parameters of the used faba bean varieties. The varieties Sakha 1 and Nubaria 2 showed higher number of pods per plant than the control following seed exposure to the dose 60 Gy while in the varieties Nubaria 3 and Giza 3, higher number of pods was attained by the dose 40 Gy. The even lower dose of 20 Gy induced the highest number of pods in the varieties Giza 843 and Giza 716. The high doses of 100 and 200 Gy of g-radiation resulted in significant reduction in shoot and root length, shoot and root fresh weight as well as shoot and root dry weight compared to the control. Higher doses also caused reduction in yield components.

These results are in agreement with Selim and El-Banna (2001) who concluded that irradiation with low doses of 5 to 50 Gy were the safe doses that can be used as a perfect tool for stimulating seed germination, growth and metabolic processes as well as increasing seed yield and the nutritional value of seeds. On the other side, doses of 100, 150 and 200 Gy were inhibitory and doses of 250 up to 400 Gy were lethal. In our results the reduction in all studied parameters in variety Giza 843 is in agreement with Artik and Peksen (2006) who also found a reduction in faba bean seed yield and harvest index in some varieties when seeds were treated with relatively low doses (25 and 50 Gy) of g-irradiation.

The exposure to g-radiations can have stimulatory effects on specific morphological traits and can increase the yield of plants in terms of growth (e.g., taller plants), reproductive success (e.g., formed seeds) and ability to withstand water shortage (Korystov and Narimanov, 1997; Maity *et al.*, 2005; Badr *et al.*, 2014). On the other hand, reduction in shoot and root length of a number of legume crops at relatively hogh doded of g-radiation, has already been reported (Veeresh *et al*. (1995; Thimmaiah *et al.* 1998; Muhammad and Afsari 2001; Toker *et al*. 2005; Kon *et al.* 2007; Badr *et al.* 2014). Soehendi *et al*. (2007) stated that modification obtained by g-irradiation in mung bean leaflet type could affect leaf canopy and alter seed yield. The present results also agree with the results of (Charumathi *et al.* 1992) on black gram, Khan *et al.* (2000) on chickpea, Yousaf *et al.* (1991) on lentil.

Svetleva and Petkova (1992) concluded that gamma irradiation had a stimulatory effect on primary branches and yield attributes in French beans (*Phaseolus vulgaris* L.) including number of pods per plant, number of flowers per plant, seed index, etc. following low doses of g-rays and inhibition of the same attributes at higher rates. They also reported inhibitory effects of g-irradiation on seed maturity, flowering, plant height, and seed yield per plant as well as prolonged growth period and retarded plant height. Preussa and Britta (2003) reported that reduction of plant height is the most frequently arising type of mutations induced by g-radiation. They find that g-radiation of uvh1plants specifically triggers a G2-phase cell cycle arrest. Mutants, termed suppressor of gamma (sog), that suppress this radiation-induced arrest and proceed through the cell cycle unimpeded were recovered in the uvh1 background; the resulting irradiated plants are genetically unstable. The sog mutations fall into two complementation groups. They are second-site suppressors of the uvh1 mutant's sensitivity to gamma radiation but do not affect the susceptibility of the plant to UV radiation. In addition to rendering the plants resistant to the growth inhibitory effects of g-radiation, the sog1 mutation affects the proper development of the pollen tetrad, suggesting that SOG1 might also play a role in the regulation of cell cycle progression during meiosis.

Although, no certain explanations for the stimulatory effects of low-dose gamma radiation are available until now, Wi *et al*., (2007) proposed that the low dose of irradiation induces growth stimulation by changing the hormonal signaling network in plant cells or by increasing the anti-oxidative capacity of the cells to easily overcome daily stress factors such as fluctuations of light intensity and temperature in the growth condition. In contrast, the high-dose irradiation that caused growth inhibition has been ascribed to the cell cycle arrest at G2/M phase during somatic cell division and/or various damages in the entire genome (Preussa and Britta, 2003). Wi *et al.* (2007) reported no significant morphological aberrations in the phenotype of plants irradiated with relatively low doses of gamma rays, while high-dose irradiation inhibited seedling growth remarkably.

In this study, seven ISSR primers gave a total of 81 amplified polymorphic fragments ranging from eight bands (primer 841) to 16 bands (primer HB12). The results revealed 6 unique bands, one is recorded in var. Nubaria 3 at the dose 100 Gy in the profile of the primer 841 with a molecular size of 700 pb, one in var. Giza 3 at the dose 20 Gy in the profile of the primer 834 with a molecular size of 1500 pb, one in var. Nubaria 3 at the dose 80 Gy in the profile of the primer 810 with a molecular size of 1000 pb, one in var. Nubaria 3 at the dose 20 Gy in the profile of the primer 807 with a molecular size of 1000 pb and two in the profile of the primer HB12, one in var. Nubaria 2 at the dose 200 Gy with a molecular size 800 pb and the other one in var. Giza 3 at the dose 60 Gy with a molecular size of 1400 pb. These results agree with the results of Abo El-Kheir *et al.* (2010) who used ISSR markers to detect genetic responses of the faba bean cultivars Giza 843, Nubaria 1 and Misr 1 to the broomrape infestation and to illustrate the effect of 25 Gy dose on the genome.

Yoko *et al*. (1996) studied the effect of gamma irradiation on the genomic DNA of corn, soybeans and wheat. They concluded that, large DNA strands were broken into small strands at low irradiation dose but small and large DNA strands were broken at higher irradiation doses. These results are in agreement with the results of Badr *et al*. (2014) who indicated an increase in polymorphism after irradiation with gamma rays of cowpea. The ISSR finger printing might be connected to structural rearrangements in DNA caused by different types of DNA damages (breaks, transpositions, deletion, etc.) as a result of the exposure of faba bean dry seeds to different doses of gamma irradiation (Sonia *et al*. 2012). They also concluded that irradiating seeds of faba bean with higher amounts of gamma rays greatly induce morphological changes. These modifications in growth traits and morphological changes were accompanied with a marked modulation in the DNA profile.

CONCLUSION

It may be concluded that, the irradiation of faba bean seeds by gamma rays at doses higher than 80 resulted in reduced growth and yield of all varieties of broad bean. The dose of 60 Gy was the most effective in improving growth traits and yield components in the two varieties Sakha 1 and Nubaria 2 and by dose of 40 Gy was effective in improving growth traits and yield in the two varieties Nubaria 3 and Giza 3. On the other hand the dose of 20 Gy was the most effective in improving the growth and yield in var. Giza 716 whereas var. Giza 834 was sensitive to all doses of g-radiation. These improvements in growth traits were accompanied with a marked modulation in DNA profile. The seven primers produced 81 bands including 75 polymorphic bands and 6 unique bands. The unique bands were scored in var. Nubaria 3 by the doses (20, 80 and 100 Gy), var. Giza 3 by the doses (20 and 60 Gy) and var. Nubaria 2 by the dose 200 Gy. The two varieties Nubaria 3 and Nubaria 2 showed higher numbers of polymorphic bands (225, 191) respectively compared to other varieties. Polymorphic information content (PIC) was estimated for each primer and ranged from 0.193 for primer 807 to 0.31 for primer 834 with a mean of 0.259.

REFRENCES

Abo El-kheir, Z.A., Abdel-Hady, M.S., El-Naggar, Hoda M.H. and Abd El-Hamed, A.R. (2010): Molecular and Biochemical Markers of Some *Vicia faba* L. Cultivars in Response to Broomrape Infestation. Nature and Science 8 (11): 252-260.

Ajebade, S.R., N.F. Weeden, S.M. Chite (2000): Inter simple sequence repeat analysis of genetic relationships in the genus *Vigna*. Euphytica 111(1): 47-55.

Alghamdi, S.S. (2009): Chemical composition of faba bean (*Vicia faba* L.) genotypes under various water regimes. P. J. of Nut. 8(4): 477-482.

Alghamdi, S.S., H.M. Migdadi, Ammar M.H., Paull J.G., Siddique K.H.M. (2012). Faba bean genomics: current status and future prospects. Euphytica. 186:609–624.

Artik, C., and Peksen, E. (2006): The effects of gamma irradiation on seed yield and some plant characteristics of faba bean (*Vicia faba* L.) in M2 generation. The J. Agri. Fac. of Ondokuz Mayıs Univ., 21(1): 95–104.

Badr, A. and El-Shazly, H.H (2012): Molecular approaches to origin, ancestry and domestication history of crop plants: barley and clover as examples. *J. Genet. Eng. Biot*, 10:1-12.

Badr, A., H. I. Sayed, M. Hamouda, M. Halawa, M. A. Elhiti (2014): Variation in growth, yield and molecular genetic diversity of M2 plants of cowpea following exposure to gamma radiation. Life Sci J. 11(8):10-19.

Bakry, B. A., T. A. Elewa, M. F. El karamany, M. S. Zeidan, M. M. Tawfik (2011): Effect of row spacing on yield and its components of some faba bean varieties under newly reclaimed sandy soil condition. W. J. Agri. Sci. 7(1): 68-72.

Belaj, A., Z.Satovic, G.Cipriani, L.Baldoni, R.Testolin, L. Rallo, I. Trujillo (2003): Comparative study of the discriminating capacity of RAPD, AFLP and SSR markers and of their effectiveness in establishing genetic relationships in olive. Theor Appl Genet. 107: 736–744.

Charumathi, M., Rao, M.V.B., Babu, R.V., and Murthy, K.B. (1992): Efficiency of early generation for induced micro-mutations in black gram (*Vigna mungo* (L.). Hepper. Nuc. Agric. Bio. 21 (4): 299–302.

Chopra V.L. (2005): Mutagenesis: investigating the process and processing the outcome for crop improvement. Curr. Sci. 89:353–359.

Dellaporta, S.L., Wood, J. and Hicks, J.B. (1983): A plant DNA minipreparation: Version Y. Plant Molec. Bio. Rep., 1:19-21.

Duc, G. (1997): Faba bean (*Vicia faba* L.). Field Crop. Res. 53: 99-109.

Duc, G; S. Bao, M. Baum; B.Redden, M. Sadiki, M. J Suso, M.Vishniakova, X. Zong (2010): Diversity maintenance and use of *Vicia faba* L. genetic resources. Field Crop. Res. 115: 270-278.

El-Danasoury, M. M., A. E. El-Ghubashy, H. Yossif , R. T. Behairy (2008): DNA fingerprinting to identify some faba bean (*Vicia faba* L.) varieties. J. Agr. Sci. Mansoura University 33(6): 4619-4630.

Ellwood SR, Phan HTT, Jordan M, Torres AM, Avila CM, Cruz-Izquierdo S, Oliver RP. (2008): Construction of a comparative genetic map in faba bean (*Vicia faba* L.); conservation of genome structure with *Lens culinaris*. BMC Genom. 9:380.

Galvan, M.Z., B. Bornet, P.A. Balatti, M. Branchard (2003): Inter simple sequence repeat (ISSR) markers as a tool for the assessment of both genetic diversity and gene pool origin in common bean (*Phaseolus vulgaris* L.). Euphytica 132: 297-301.

Gilbert, J. E., R. V. Lewis, M. J. Wilkinson, P. D. S. Caligari (1999): Developing an appropriate strategy to assess genetic variability in plant germplasm collections. Theo. App. Genet. 98: 1125-1131.

Gonzales, A., W. A. Delgado-Salinas, R. Papa, P. Gepts (2005): Assessment of inter simple sequence repeat markers to differentiate sympatric wild and domesticated populations of common bean. Crop Sci. 45: 606-615.

Jaywardena SDL, Peiris R (1988): Food crop breeding in Srilanka. Arch. and Challe.. Biol. N., 2: 22-34.

Khalafallah, A. A., K. M. Tawfik, Z. A. Abd El- Gawad (2008): Tolerance of seven faba bean varieties to drought and salt stresses. Res. J. of Agri. & Biol. Sci. 4 (2): 175-186.

Khan, M.R., Qureshi, A.S., and Hussain, S.A. (2000): Gamma irradiation sensitivity and its modulation with gibberellic acid for seedling physiology in chickpea (*Cicer arietinum* L.). Proc. of P. Aca. of Sci. 37(2): 195–202.

Kon, E., O.H. Ahmed, S. Saamin and N.M. Majid, (2007): Gamma radiosensitivity study on long bean (*Vigna sesquipedalis*). Am. J. Applied Sci., 4(12): 1090-1093.

Korystov, Y.N., and Narimanov, A.A. (1997): Low doses of ionizing radiation and hydrogen peroxidase stimulate plant growth. Biologia (Bratisl.), 52: 121–124.

Link, W., C. Dixkens, M. Singh, M. Schwall, A. Melchinger (1995): Genetic diversity in European and Mediterranean faba bean germplasm revealed by RAPD markers. Theo. and App. Gen. 90: 27-32.

Maity, J.P., Mishra, D., Chakraborty, A., Saha, A., Santra, S.C., and Chanda, S. (2005): Modulation of some quantitative and qualitative characteristics in rice (*Oryza sativa* L.) and mung (*Phaseolus mungo* L.) by ionizing radiation Radiat. Phys. Chem. 74(5): 391– 394.

Maluszynski, M., Nichterlein, K., van Zanten, L. and Ahloowalia, B.S. (2000): Officially Released Mutant Varieties-The FAO/IAEA Database. Mut. Breed. Rev. 12:1−84.

Muhammad, R. and S. Afsari, (2001): Quantitative variations induced by gamma irradiation and gibberellic acid in M1 generation of Chick pea. Sarhad J. Agric., 17(3): 367-372.

Porebski, S., Grant Bailey, L. and Baum, B.R. (1997): Modification of a CTAP DNA extraction protocol for plants containing high polysaccharide and polyphenol components. Pl. Mol. Bio. Rep, 15 (1): 8-15.

Preussa, S.B., and Britt, A.B. (2003): A DNA-damage-induced cell cycle checkpoint in *Arabidopsis*. Genetics, 164: 323–334.

Reddy, M. P., N. Sarla , E. A. Siddiq (2002): Intersimple sequence repeat (ISSR) polymorphism and its application in plant breeding. Euphytica 128: 9-12.

Selim A., El-Banna (2001): Ionizing irradiation effects on germination, growth, some physiological and biochemical aspects and yield of pea plants. Umweltverschmutzung in Ägypten: Folgen für Mensch, Tier und Pflanze Symposium, 29 Sept.-3 Oct. Cairo, Ägypten.

Singh B.D. (2005): Mutations in crop improvement. In: Singh BD (ed) Plant breeding, principles and methods. Kalyani Publishers. Ludhiana, pp 698–731.

Soehendi, R., Chanprame, S., Toojinda, T., Ngampongsai, S., and Srinives, P. (2007): Genetics, agronomic, and molecular study of leaflet mutants in mungbean (*Vigna radiata* (L.) Wilczek). J. Crop Sci. Biotech. 10(3): 193–200.

Sonia M, Yassine,Marie V,PhilippeD,Philippe S, Mouldi S.Omrane B. (2012): Variation in quantitative characters of faba bean after seed irradiation and associated molecular changes. Afri, J, Biotechn. 11(33):. 8383-8390.

Sudupak, M. A. (2004): Inter- and intra-species inter simple sequence repeat (ISSR) variation in the genus *Cicer*. Euphytica 135: 229-238.

Svetleva, D., and Petkova, S. (1992): Association between changes in the M1 and mutability in the M2 in the French bean variety 564 after combine treatment with g-radiation and N-allyl- Nnitrosourea. Genetika i Selektsiya;, 25: 254–260.

Terzopoulosa, P. J., P. J Bebeli (2008): Genetic diversity analysis of Mediterranean faba bean (*Vicia faba* L.) with ISSR markers. Field Crop. Res. 108: 39-44.

Thimmaiah, S.K., P. Mahadevu, K.N. Srinivasappa and A.N. Shankra, (1998): Effect of gamma irradiation on seed germination and seedling vigor in cowpea. J. Nuclear Agric. And Biol., 27(2): 142-145.

Toker, C., B. Uzun, H. Canci, F. Oncu Ceylan. (2005): Effects of gamma irradiation on the shoot length of *Cicer* seeds. Radiat. Phys. Chem., 73: 365-367.

Torres AM, Avila CM, Gutiérrez N, Palomino C, Moreno MT, Cubero JI. (2010) Marker-assisted selection in faba bean (*Vicia faba* L.) Field Crop Res115:243–252.

Veeresh, L.C., G. Shivashankar, H. Shailaga and S. Hittalmani (1995): Effect of seed irradiation on some plant characteristics of winged bean. Mysore J. Agric. Sci., 29: 1-4.

Wi, S.G., B.Y. Chung, J.S. Kim and *et al*. (2007): Effects of gamma irradiation on morphological changes and biological responses in plants. *Micron*, 38: 553-564.

Yoko K, Aya M, Hiromi I, Takashi Y, Kukio S (1996): Effect of gamma irradiation on cereal DNA investigated by pulsed-field gel electrophoresis. Shokuhin-Shosha 31: 8-15.

Yousaf, H., Raziuddin, , and Ahmad, H. (1991): Morphology and chemical studies of irradiated lentil (*Lens culinaris* Med.). Sarhad J. Agric. 7: 361–368.

Zeid, M., S. Mitchell, W. Link, M. Carter, A. Nawar, T. Fulton, S. Kresovich (2009): Simple sequence repeats (SSRs) in faba bean: new loci from *Orbanche* resistant cultivar 'Giza 402'. Plant breeding 128(2):149-155.

Zied, M., C. C. Schon, W. Link (2003): Genetic diversity in recent elite faba bean lines using AFLP markers. Theo. and App. Gen.107: 1304-1314.

Zietkiewicz, E., Rafalski, A., & Labuda, D. (1994). Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics, 20(2), 176-183.