

## Effect of Various Doses Gamma-radiation on Phenotypic and Molecular Characteristics of Two Egyptian Soybean Varieties

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**T**WO Egyptian soybeans varieties, Giza111 and Crawford were selected for the current study. Their seeds were exposed to three doses of gamma-radiation 150, 200, and 250 Gy in order to study their mutagenic effects in the treated seeds and two following generations at various levels. In the first generation (M1), two doses (150 and 200 Gy) were the most effective in enhancement of some agronomic traits like number of stem branches, number of pods per plant, number of seeds per pod and weight of 100 seeds and crop yield. Analysis of protein profiles using SDS-PAGE showed variation among the treatments in each variety, where a total of 35 protein bands were recorded. Three of which were unique bands, one unique band of size 37 kDa was found in the treated seeds before germination (M0) in Crawford at 250 Gy treatment. The other two unique bands (102 and 162 kDa) were found in M2 generation in Crawford at 150 Gy and Giza111 at 200 Gy, respectively. ISSR analysis using five primers gave a total 45, 38 and 28 ISSR bands in M0, M1 and M2, respectively; 4 of which were unique bands. The primer HB14 amplified 6 DNA bands, 2 were unique bands (895 & 1050 bp), both in M1 generation of Giza111 at 150 Gy, while primer 844 and 862 amplified only one unique band both found in M0 Giza111 control. They have a molecular size of 888 and 900 bp, respectively. Although, UBC-827 amplified the highest number of bands (7) no unique bands were found.

**Keywords:** *Glycine max* L., Gamma-radiation, SDS-PAGE, ISSR, polymorphism.

Soybean (*Glycine max* L.) integrates in one crop both the dominant world supply of edible vegetable oil, and the dominant supply of high-protein feed supplements for livestock. Its seed residues and derivatives have a substantial economic importance in a wide range of industrial, food, pharmaceutical, and agricultural products (Smith and Huyser, 1987). In addition, soybean protein is rich in the valuable amino acid lysine (5%) in which most of the cereals are deficient. Moreover, it contains a good quantity of minerals, salts and vitamins. Soybean's oil has been used in Egypt since year 1976, its oil extraction ratio is about 20.5%. Furthermore, soybean is an essential source of protein in the animal and poultry feed (Çelik *et al.*, 2014).

Mutations, both spontaneous and induced have been highly successful in changing the fatty acid composition of several oil seed crops. Three reasons have been given for attempts in changing seed oil quality by means of single gene mutations being exceptionally successful. Various soybean mutant lines were identified using mutation, which possessed many desirable traits like habit, low linolenic, high oleic content and resistance to pod shattering (Rahman *et al.*, 1995). In addition, mutational breeding is thought to be as a source of increasing genetic variability and particular improvement could be conferred without importantly changing its satisfactory phenotype (Mudibu *et al.*, 2011; Ambavane *et al.*, 2015). In plants, induction of genetic and morphological variabilities, using induced mutation, has been found as the most efficient method. Several studies have shown that exposure to gamma-radiations can have stimulatory effects on specific morphological parameters and can increase the yield of plants and their resistance to drought (Yu *et al.*, 2007; Melki and Dahmani, 2009; Hanafiah *et al.*, 2010; Badr *et al.*, 2014a and b; Ariraman *et al.*, 2016 and Gaafar *et al.*, 2016). One of the most successful mutagenic agents is radiation, which induced mutation breeding in various crops and ornamental plants (Schum, 2003). Radiation represented excellent means of stimulating the expression of recessive genes, thus inducing new genetic variation (Song and Kang, 2003).

The effectiveness of gamma radiation in improving plant growth, seed quality, cooking time and physiological processes is highly related to the level of doses used (Lima *et al.*, 2011; Hegazi and Hamideldin, 2010) showed that gamma radiation at 0.4 kGy showed the highest improvement in okra plant growth and seed yield when compared to 0.3 kGy and 0.5 kGy. However, the application of gamma radiation in developing high grain yield soybean varieties was limited (Lima *et al.*, 2011). Addai and Safo-Kantanka (2006) subjected three soybean genotypes to (0, 50, 100, 150, 200, 250 and 300 Gy) of gamma radiation. It has been found that emergence percentage and seedling height have been approximately reduced to 50% by 250 Gy dose compared to the control (0 Gy). Therefore, it was considered as the appropriate dose for induced mutation in the used genotypes. Variations in M1 generation, though less important in view of obtaining stable gene mutations, are often considered as indicator in measuring efficiency of the mutagens (Plesnik, 1993). Meanwhile, various studies were dealing with analysis of M2 and M3 generations of soybean induced by physical and chemical mutagens (Patil *et al.*, 1985).

Many molecular marker systems have been routinely used to evaluate mutations and genetic variation in plants. These include SDS-PAGE protein patterns, randomly amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs) and inter simple sequence repeats (ISSRs) (Atienzar and Jha, 2006). Afify and Shousha (1988) described that changes in protein subunit patterns were identified in the cultivar "Clark" using SDS-PAGE,

depending on the dose of  $\gamma$ -rays, where three high molecular weight protein bands were found in soybean cultivars exposed to  $\gamma$ -rays. Moreover, it has been shown that radiation caused native protein aggregation, which might simply have resulted from rearrangement of the composed protein subunits. ISSR is a technique that is widely used for assessing the alteration in DNA sequences induced by mutagenic agents such as gamma rays (Mudibu *et al.*, 2011 and Mejri *et al.*, 2012; Hamideldin and Eid, 2015; Gaafar *et al.*, 2016). Currently, induced mutation is widely used for introducing of genes, which control important traits and understanding the function and mode of action of such genes as well as developing improved crop varieties.

The aim of this study is to evaluate the effect of distinct doses of gamma rays on characteristics, protein profiles and ISSR polymorphism of two soybean varieties, in order to identify gamma-induced mutant lines with potentially improved agronomic characteristics.

### Material and Methods

Seeds of the two soybean varieties (Giza 111 and Crawford) were obtained from the Food and Legumes Research Department, Field Crops Research Institute, Agricultural Research Center, Giza, Egypt in 2013. Dry seeds of the two soybean varieties were exposed to three different doses of gamma radiation at the Atomic Energy Center, Nasr City, Cairo, Egypt using cobalt 60 as a source of gamma-radiation. The applied doses were 150, 200, and 250 Gy. The seeds of control samples were not exposed to gamma radiation.

#### *Morphological analysis*

In season 2014, exposed and control seeds of the two soybeans varieties were grown to maturity in the greenhouse of Botany Department, Faculty of Science, Tanta University. Forty soybean gamma-radiated seeds (treated seeds parents or M0 generation) of each dose/variety as well as controls were cultivated per line. Then after four weeks of growth, the seedlings were then thinned to 20 plants per line. Plants were irrigated every 10 to 14 days from sowing until maturity and 20 lb/acre of phosphate fertilizer was employed as a side band with the seed and at seedling and fruiting stages.

Morphological observations were carefully and continuously recorded throughout the stages of growth in order to examine the morphological variation in M1 plants compared to the control. A number of morpho-agronomic parameters were measured after six weeks from sowing. The measured criteria were seedling length (cm), days to flowering, days to maturity, shoot length (cm), number of branches of stem, number of pods per plant, number of seeds per pod, pod length (cm), first germination percentage (%), seed length (cm),

weight of 100 seeds (gm), crop yield (no. seeds/plant), and pod shattering (%). In addition, presence of stem appendages, leaf abnormalities, flower color, pod appendages, pod color, coat color, seed hilum color were also documented.

In season 2015, the seeds of the M1 plants were then sown and grown as explained above. In this field experiment, all morphological measurements were also recorded for the M2 plants as previously described above.

#### *Protein analysis*

Total seed proteins were extracted using 0.03 M Tris-HCl pH = 8 as an extraction buffer, 0.01 gm of the dry seed was grinded in a mortar with pestle, then 400 µl of the extraction buffer was added. The extraction was done in Eppendorf tubes over night at 4°C. After that the tubes were centrifuged at 15000 rpm for 20 min at 4°C (Sigma 3K 18 Bench Top centrifuge). Main slab gel of 12% polyacrylamide (7.5 x 6 x 0.1 cm) was prepared as described by Laemmli (1970). Molecular weight marker proteins (Fermentas, Germany) run in parallel to samples were BSA (66 kDa), egg albumin (45 kDa), glyceraldehyde-3-phosphate dehydrogenase (36 kDa), carbonic anhydrase (29 kDa), trypsinogen (24 kDa), trypsin inhibitor (20.1 kDa), and α-lactalbumin (14.2 kDa). 50 µl of the protein extracts were applied into the PAGE gel wells. The electrophoresis was carried out at 100 V and 15 mA using power supply (DYY-12 power supply Beijing Liuyi, Germany) until the stacking gel was ended, then completed at 150 V and 20 mA in 1 × running protein buffer until reached the bottom of the gel. The bands were stained overnight with coomassie brilliant blue (CBB) solution, then destained using the same solution without CBB until the gel background became clear.

#### *DNA extraction*

For DNA extraction, the indirect DNA extraction method (Gene JET DNA purification kit Molecular Biology, Thermo Scientific Company, Germany) was used, 0.1 gm of dry seed were grounded and extracted with 350 µl of lysis buffer A, the tubes were properly vortexed for 20 secs, and 50 µl lysis buffer B was added. After that, 20 µl of RNase was added and the extract was incubated in a shaking water bath at 65°C for 10 min. The tubes were then vortexed and incubated on ice for 5 min and centrifuged at 14000 rpm for 5 min. The supernatants were transferred into clean Eppendorf tubes, after that 400 µl of plant DNA binding solution and 400 µl of 96% ethanol were added to the column, so the volume became 1300 µl, then half of the volume was loaded into the column and centrifuged at 8000 rpm for 1 min. and the supernatant was thrown, repeat this step for the other half, then the columns were washed with 500 µl of washing buffer 1, and centrifuged for one min. at 8000 rpm and the supernatant was thrown. Subsequently, 500 µl of washing buffer 2 was added and columns were centrifuged for 3 min at 14000 rpm, and the supernatant was

thrown. Extra centrifugation step was done for 1 min at 14000 rpm. The DNA was eluted by adding 100 µl elution buffer, the columns were kept at room temperature for 5 min, then centrifuged for 1 min at 10000 rpm. The centrifugation step was repeated two times and then the extracted DNA was stored in -80 °C till use.

#### *ISSR amplification and electrophoresis*

In total, 12 ISSR primers (Operon Nippon EGT CO. LTD.USA), were screened for the production of polymorphic products from all samples. Only five primers showed polymorphic patterns and were selected. This was done after amplification conditions were optimized by selecting appropriate MgCl<sub>2</sub> concentrations and anneal temperatures (Table 1).

**TABLE 1. Names, sequences and annealing temperatures of the selected ISSR primers.**

Primer name	Sequence	Annealing temp.
<b>862</b>	5'-AGCAGCAGCAGCAGCAGC-3'	57.5
<b>UBC-827</b>	5'-ACACACACACACACACG-3'	41
<b>HB10</b>	5'-GAGAGAGAGAGACC-3'	44
<b>844</b>	5'-CTCTCTCTCTCTCTGC-3'	38.8
<b>HB14</b>	5'-GTGTGTGTGTGTCC-3'	44

Isolated genomic DNA was used as template in the amplification reactions. A total of 25 µl, with 12.5 µl green master mix reaction mix was prepared (NIPPON Genetics EUROPE Master mix Taq Ready Mix with Dye), 3 µl primer of concentration 10 pmole/µl. Amplification conditions were optimized using a gradient thermal cycler (Biometra Thermal Cycler, Germany). The following PCR program with following settings was used as described by Gaafar et al. (2015): an initial denaturation at 94°C for 4 min., followed by 40 cycles of 45 sec. at 94°C for denaturation, annealing temperature, based on each primer composition, for 45 sec., and 45 sec. at 72°C for extension and a final extension at 72°C for 7 min., and then temperature was set at 4°C till the removal of PCR tubes. The PCR products were separated on 1.5 % agarose gel by mixing 12.5 µl of the PCR-products of each primer with 2.5 µl of loading buffer and loading into the agarose wells. Gel electrophoresis was performed in 0.5 × TAE buffer at 70 V for 1 hr ISSR gels were visualized using UVP gel documentation system (UVP, UK) and photographed under UV light. The size of each band was estimated using 100 bp DNA ladder (Fermentas, Germany) as a standard marker. Because ISSRs are multi-loci and dominant markers, the amplified polymorphic DNA fragments were scored as binary presence (1) or absence (0), and the percentage of polymorphic fragments was used to evaluate the genetic variability and mutations in the two used soybean varieties.

*Statistical analysis*

Statistical presentation and analysis of the quantitative traits was conducted by calculating the mean, standard deviation (SD) and analysis of variance (ANOVA) tests using the COSTAT analysis of variance (ANOVA). One-way test was used to compare P and LSD values among different varieties and gamma doses in the same criteria of the quantitative morphological measurements.

**Results and Discussion***Morphological analysis*

At morphological level, the 150 and 200 Gy doses were the most effective in increasing some of these morphological characters from M1 to M2 like germination percentage, shoot length, stem branching, pod shattering, crop yield (no. of seeds/plant), number of pods/plant, pod length, number of seeds/pod and weight of 100 seeds (Table 2a and 2b).

**TABLE 2a. Mean of the quantitative morphological characteristics of M1 generation of the two soybean varieties (Giza111 and Crawford) after exposure to different doses of gamma-irradiation.**

Character	Soybean variety							
	Control	Giza111			Control	Crawford		
		150 Gray	200 Gray	250 Gray		150 Gray	200 Gray	250 Gray
Days to flowering	30	26	35	35	31	31	32	80
Days to maturity	55	64	80	80	50	60	76	109
Germination (%)	100	75	25	67.5	50	47.5	15	10
Pod shattering (%)	1.68	9.1	6.8	1.2	2.9	2.8	0	0
Weight of 100 seeds (g)	15	14.5	13	12.5	13	10.14	1.85	0.8
No. of seeds per plant	3.26	1.83	2.42	1.7	2.86	1.96	1.3	1.6
No. of pods per plant	87	47	27	31	74	25	6	2.5
Days to flowering	30	26	35	35	31	31	32	80
Days to maturity	55	64	80	80	50	60	76	109

**TABLE 2b. Mean of the quantitative morphological characteristics of M2 generation of the two soybean varieties (Giza111 and Crawford soybeans) after exposure to different doses of gamma-irradiation.**

Character	Soybean variety							
	Control	Giza111			Control	Crawford		
		150 Gray	200 Gray	250 Gray		150 Gray	200 Gray	250 Gray
Days to flowering	30	36	36	40	28	36	36	0
Days to maturity	55	83	80	90	50	64	50	0
Germination (%)	35	20	40	45	67.6	12.5	15	7.5
Pod shattering (%)	5.9	0	2.7	4.7	5.7	8.5	5	0
Weight of 100 seeds (g)	13.4	12.3	14.5	14	12	11	10.5	0
No. of seeds per plant	3.1	2.6	3	2.6	2.8	2.6	2.8	0
No. of pods per plant	34	34	36.1	35.1	65.6	75.4	42.5	0
Days to flowering	30	36	36	40	28	36	36	0
Days to maturity	55	83	80	90	50	64	50	0

In Giza111 and Crawford M1 plants, seedling height, number of seeds per pod and stem branching, decreased gradually depending on increase in gamma radiation (150, 200 and 250 Gy), the same was found in M2 plants as well. Reduction in seed yield (seeds/plant) in M1 generation was observed in Crawford after exposure to dose 250 Gy. This reduction was in accordance with what have been found by Mehetre *et al.* (1994) who confirmed that yield reduction was resulting from injury effect in seeds and usually showed inhibitory effects on seeds of angiosperms and gymnosperms (Thapa, 1999). Our results are in line with Sharif *et al.* (2000), Din *et al.* (2003), Kon *et al.* (2007), and Marcu *et al.* (2013). Comparison of yield (seeds/plant) of the two varieties under study revealed that Giza111 seed yield (seeds/plant) is higher than Crawford seed yield (seeds/plant) in all related doses. These results might be due to stimulating effects of gamma-radiation on activating RNA synthesis or protein synthesis (Kuzin *et al.*, 1976).

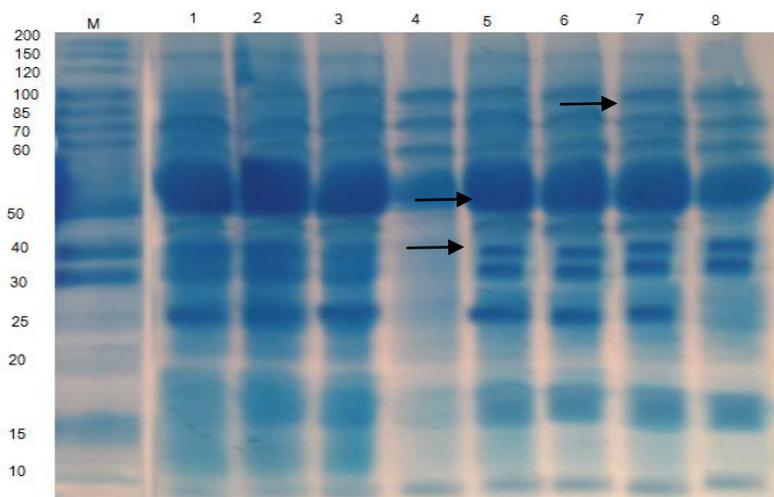
In study, the 250 Gy dose had increased slightly some characters like shoot height, number of pods per plant, pod length, percentage of germination, seed length and absence of leaf abnormalities from M1 to M2 generations. The 250 Gy dose in Crawford showed very low percentage of seed germination because the plants with high dose of radiation didn't give mature seeds. This was also reported by Ahmad and Qureshi (1992) in *Zea mays* L. and Din *et al.* (2003) in *Triticum aestivum* L. Our results also showed a decrease in shoot and root length when high gamma radiation dose (250 Gy) was used. This observation could be correlated with the results obtained by Khalil *et al.* (1986), who attributed a decreased in shoot and root lengths at higher doses of gamma rays resulted from reduction mitotic activity in meristematic tissues and reduced moisture contents

in seeds. The decrease in morphological characteristics of various crops has already reported by (Thimmaiah *et al.*, 1998; Khan and Qureshi, 2001; Al-Salhi *et al.*, 2004 and Toker *et al.*, 2005), which supports the present investigation.

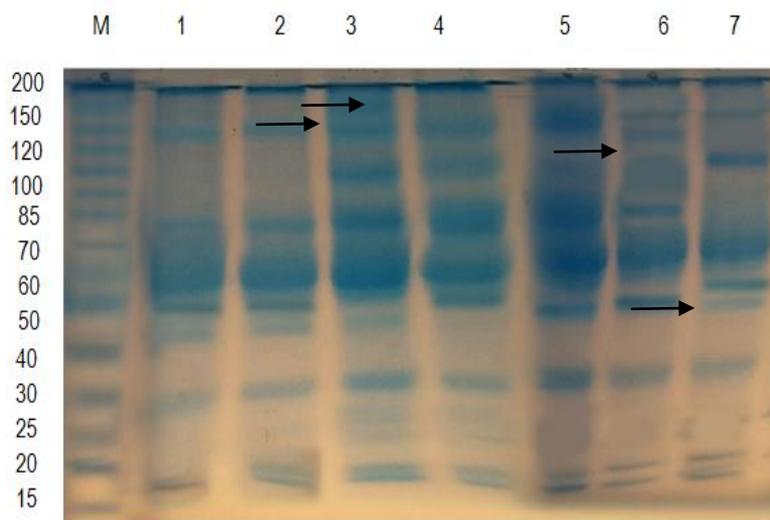
#### *Protein analysis*

After treatment of the two soybean varieties (Giza 111 and Crawford) with different doses of gamma rays (150, 200 and 250 Gy), variation in protein banding patterns was studied using SDS-PAGE for each variety and in treated seeds and the two following generations (M0, M1 and M2) (Fig. 1a & 1b and Table 3).

All living organisms are normally affected with radiation, which is a crucial environmental stress. Particularly, cellular components are usually affected with the ionizing radiation and thus inducing biochemical and molecular changes in plants (Vanhoudt *et al.*, 2010). Evaluation of these biological changes is very important as they occur through such biochemical changes. Ionizing radiation produces OH<sup>•</sup> and O<sub>2</sub><sup>•-</sup>, which induced damage in DNA and proteins by breaking chemical bonds in such biomolecules (Roldán-Arjona and Ariza, 2009; Esnault *et al.*, 2010; Somosy, 2010 and Çelik *et al.*, 2014).



**Fig. 1a.** Protein profiles of M1 generation seeds of the two soybean varieties (Giza111 and Crawford) using SDS-PAGE technique. 1=Giza111 control, 2= Giza111 treated with 150 Gray, 3= Giza111 treated with 200 Gray, 4= Giza111 treated with 250 Gray, 5= Crawford control, 6= Crawford treated with 150 Gray, 7= Crawford treated with 200 Gray and 8= Crawford treated with 250 Gray.



**Fig. 1b.** Protein profile of M2 generation seeds of the two soybean varieties (Giza111 and Crawford) using SDS-PAGE technique. 1=Giza111 untreated control, 2= Giza111 treated with 150 Gray, 3= Giza111 treated with 200 Gray, 4= Giza111 treated with 250 Gray, 5= Crawford untreated control, 6= Crawford treated with 150 Gray and 7= Crawford treated with 200 Gray.

**TABLE 3.** Summary of the observed protein bands in SDS-PAGE patterns of the M0, M1 and M2 generations of the two soybean varieties (Giza111 and Crawford).

Generation		Soybean variety	
Type of band		Giza111	Crawford
<b>M0</b>	M	11	11
	P	2 (16 kDa / 250 Gy)	2 (16 kDa / 250 Gy)
	U	0	1 (37 kDa / 250 Gy)
<b>Total</b>		<b>13</b>	<b>14</b>
<b>M1</b>	M	5	9
	P	7	2
	U	0	0
<b>Total</b>		<b>12</b>	<b>11</b>
<b>M2</b>	M	5	6
	P	4	4
	U	1 (162 kDa / 200 Gy)	1 (102 kDa / 150 Gy)
<b>Total</b>		<b>10</b>	<b>10</b>

Gy = Gray, M = monomorphic, P = polymorphic, U = unique, M0= irradiated seeds before planting, M1= first mutant generation, M2= second mutant generation.

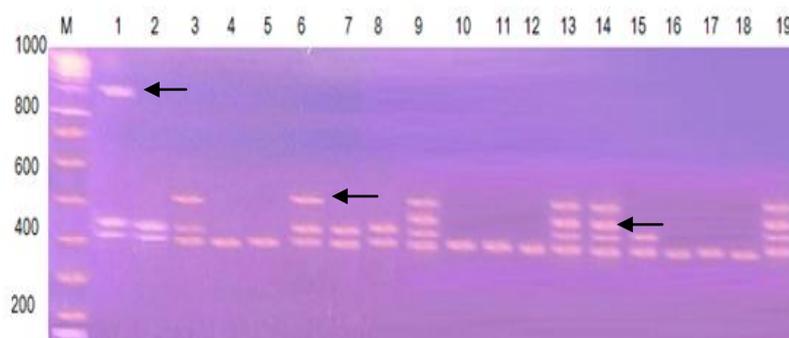
Total of 35 protein bands were found. Three of which were unique protein bands, one unique band (37 KDa) was found in M0 in Crawford at 250 Gy treatment. The other two unique bands (102 and 162 KDa) were found in M2 generation in Crawford at 150 Gy and Giza111 at 200 Gy, respectively (Fig. 1a and 1b; Table 3). This clearly indicated that gamma rays have induced changes in gene expression leading to change in types of the translated proteins in the SDS protein profiles of the studied soybean varieties. However, Kiong *et al.* (2008) reported no significant change in protein profiles after gamma-irradiation.

Interestingly, the results showed that there is an appearance and disappearance of protein bands in the seed protein profiles after exposure to the  $\gamma$ -radiation (Fig. 1a and 1b). These changes may be caused by denaturation of protein, protein association or deamination as described by Abu *et al.* (2005). Moreover, the appearance and disappearance of protein bands may be related to environmental stresses that cause changes in gene expressions (Ariraman *et al.*, 2016). It has been found that gamma-radiation produces active species such as hydrated electrons and hydroxyl radicals (OH $\cdot$ ) by its action on the water molecules, which successively react with the protein molecules as described by Yamamoto (1992). In addition, the chemical changes stimulated by irradiation in proteins include, degradation, cross-linking, disruption of the ordered structure of protein molecules and aggregation of the polypeptide chains caused by oxygen radicals as well (Gaber, 2005).

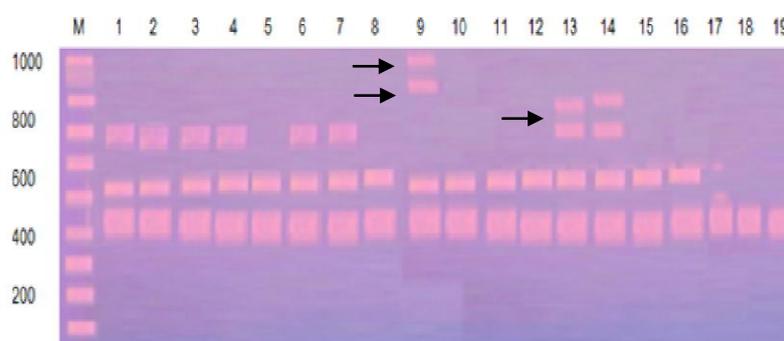
The results, shown in this study, are in agreement with Lee *et al.* (2005), who showed that low dose of gamma-irradiation was not effectual in producing of proteins with high molecular weights in soybean. Gamma-radiation at low dose caused slight breakdown of polypeptide chains and therefore, less formation of high molecular weight proteins. In case of higher gamma doses (200 and 250 Gy), there were cross-linked products of the degraded protein molecules that could not penetrate the PAGE gel (Fig. 1b).

#### *ISSR analysis*

DNA-based makers are the most reliable tools for evaluating the genetic variation because they are not influenced by environmental conditions. ISSR is a widely-used technique for assessing mutations in DNA sequences, which are induced by mutagens such as gamma radiation. Mudibu *et al.* (2011) showed that ISSR is a useful marker type that used for identifying polymorphisms in DNA sequences. In this study, five primers gave a total 45, 38 and 28 ISSR bands in M0, M1 and M2, respectively. 25, 18 and 16 of which were polymorphic in M0, M1 and M2, respectively, 4 were unique bands and 19, 18 and 16 were monomorphic bands in M0, M1 and M2, respectively. The primer HB14 amplified 6 DNA bands, 2 were unique bands (895 & 1050 bp), both in M1 generation of Giza111 at 150 Gy, while primer 844 and 862 amplified only one unique band both found in M0 Giza111 control. They have a molecular weight of 888 and 900 bp, respectively. Although, UBC-827 amplified the highest number of bands (7 ISSR bands in M0) no unique bands were found (Fig. 2a and 2b).



**Fig 2a.** ISSR banding patterns of M0, M1 and M2 generations of the two soybean varieties (Giza111 and Crawford) using 844 primer. 1=Giza111 control. From 2 to 4 are Giza111 M0 treated with 150, 200 & 250 Gray, respectively. 5= Crawford control. From 6 to 8= Crawford M0 treated 150, 200 & 250 Gray, respectively. From 9 to 11= Giza111 M1 of M0 treated with 150, 200 & 250, respectively. From 12 to 14= Crawford M1 of M0 treated with 150, 200 & 250 Gray, respectively. From 15 to 17= Giza111 M2 of M0 treated with 150, 200 & 250 Gray, respectively. From 18 to 19= Crawford M2 of M0 treated with 150 & 200 Gray, respectively.



**Fig. 2b.** ISSR banding patterns of M0, M1 and M2 generations of the two soybean varieties (Giza111 and Crawford) HB14 primer. 1=Giza111 control. From 2 to 4 are Giza111 M0 treated with 150, 200 & 250 Gray, respectively. 5= Crawford control. From 6 to 8= Crawford M0 treated 150, 200 & 250 Gray, respectively. From 9 to 11= Giza111 M1 of M0 treated with 150, 200 & 250, respectively. From 12 to 14= Crawford M1 of M0 treated with 150, 200 & 250 Gray, respectively. From 15 to 17= Giza111 M2 of M0 treated with 150, 200 & 250 Gray, respectively. From 18 to 19= Crawford M2 of M0 treated with 150 & 200 Gray, respectively.

Disappearance of ISSR bands in some M1 and M2 plants (Fig. 2a and 2b; Table 4) could be referred to damages of DNA like single or double-strand breaks, modified bases, oxidized bases, and bulky adducts. In addition, DNA-protein cross links, point mutations and rearrangement of chromosomes induced by gamma irradiation (Ginchner *et al.*, 2008 and Abhay Raj *et al.*, 2014). Moreover, it has been shown that free radicals interact with biomolecules as DNA and remove electrons from them, which in turn causes damage to both structure and activity of the DNA. During ISSR amplification, as Taq polymerase reaches a DNA damage, there could be a blockage by potential dissociation of the enzyme-DNA, which will cause the loss of ISSR bands. However, the appearance of new bands could be related to the effect of mutation instead of DNA damage (Atienzar *et al.*, 2006).

Yoko *et al.* (1996) have examined the effect of gamma-irradiation on the genomic DNA of corn, soybean and wheat plants. Their results showed that the large strands of DNA were broken into small strands at low irradiation dose. However, small and large DNA strands were broken at higher irradiation doses. Moreover, Kahrizi *et al.* (2012) showed that when gamma doses were increased, the nuclear DNA content was decreased in all the genotypes studied. Treating plants with highest doses of gamma rays resulted in the highest decrease in content of genomic DNA. Similarly, the appearance or disappearance of bands under gamma irradiation might be considered as molecular markers for radiation processes (Hussein, 2012). It has been exhibited that effects of gamma-rays on ISSR fingerprinting might be connected to structural rearrangements in DNA caused by different types of DNA damages (Mejri *et al.*, 2012) (Fig. 2a and 2b; Table 4).

### Conclusion

Applying of gamma radiation has a reduction effect on yield and other morphological characteristics of two soybean varieties. This was clear particularly with the high gamma-ray doses. In addition, gamma-radiation at low dose caused slight breakdown of polypeptide chains and therefore, less formation of high molecular weight proteins. Furthermore, there was an appearance and disappearance of protein bands in the seed protein profiles after exposure to the  $\gamma$ -radiation as well as in ISSR profiles, which might be caused by different types of DNA damages.

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**TABLE 4. Summary of amplified ISSR fragments using five selected ISSR primers in the M0, M1 and M2 generations of the two soybean varieties (Giza111 and Crawford).**

		Soybean variety									
		Giza111					Crawford				
Generation	Type of band	844	862	HB10	HB14	UBC-827	844	862	HB10	HB14	UBC-827
<b>M0</b>	<b>M</b>	1 (419 bp)	1 (452 bp)	1 (269 bp)	3	3	2	1 (452 bp)	1	2	4
	<b>P</b>	2	4	4	0	4	1	2	4	1	3
	<b>U</b>	1 (888 bp)	1 (900 bp)	0	0	0	0	0	0	0	0
<b>Total</b>		<b>4</b>	<b>5</b>	<b>5</b>	<b>3</b>	<b>7</b>	<b>3</b>	<b>3</b>	<b>5</b>	<b>3</b>	<b>7</b>
<b>M1</b>	<b>M</b>	1	2	(269 bp)1	2	3	1	1	2	2	3
	<b>P</b>	3	0	4	0	2	3	3	0	2	1
	<b>U</b>	0	0	0	2	0	0	0	0	0	0
<b>Total</b>		<b>4</b>	<b>2</b>	<b>5</b>	<b>4</b>	<b>5</b>	<b>4</b>	<b>4</b>	<b>2</b>	<b>4</b>	<b>4</b>
<b>M2</b>	<b>M</b>	1	2	1 (269 bp)	1	3	1	3	1 (269 bp)	1	3
	<b>P</b>	1	2	3	2 (1050 & 895 bp / 150 Gy)	1 (949 bp)	3	3	0	0	1
	<b>U</b>	0	0	0	0	0	0	0	0	0	0
<b>Total</b>		<b>2</b>	<b>2</b>	<b>4</b>	<b>3</b>	<b>4</b>	<b>4</b>	<b>3</b>	<b>1</b>	<b>1</b>	<b>4</b>

**Gy** = Gray, **M** = monomorphic, **P** = polymorphic, **U** = unique, **M0**= irradiated seeds before planting, **M1**= first mutant generation, **M2**= second mutant generation.

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## تأثير جرعات مختلفة من أشعة جاما على الصفات الظاهرية والجزئية لصنفين مصريين من فول الصويا

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يعتبر فول الصويا هو أحد أهم المحاصيل غذائياً وتجارياً. في هذه الدراسة تم إختيار صنفين مصريين من فول الصويا وهما جيزة ١١١ وكراوفورد وتعرضهما لثلاث جرعات مختلفة من أشعة جاما (١٥٠ ، ٢٠٠ ، و ٢٥٠ جراي) وذلك لدراسة تأثيرها المطفر على مدار ثلاثة أجيال متتالية. في الجيل الاول M1 ، وجد أن الجرعتين ١٥٠ و ٢٠٠ جراي نتج عنهما زيادة في بعض الصفات الظاهرية مثل عدد الأفرع في الساق ، الإنتاجية ، عدد القرون لكل نبات ، عدد البذور لكل قرن و وزن ١٠٠ بذرة.

وقد تم تحليل بروتينات البذور باستخدام التفريد الكهربائي لهلام الاكريلاميد ، واطهرت النتائج تنوع عالي في أنماط البروتينات للمعاملات المختلفة لكل صنف. حيث ظهرت ٣٥ حزمة بروتينية منهم ثلاثة من النوع الفريد ، وكان الوزن الجزئي للبروتين الاول هو ٣٧ كيلو دالتون وظهر في الصنف كراوفورد المعامل بجرعة الإشعاع ٢٥٠ جراي في الجيل M0 ، بينما كان الوزن الجزئي للبروتينين الأخرين ١٠٢ و ١٦٢ كيلو دالتون وظهر في الجيل الثاني M2 في الصنف كراوفورد المعامل بالجرعة ١٥٠ جراي والصنف جيزة ١١١ المعامل بالجرعة ٢٠٠ جراي.

وباستخدام تحليل تكرارات التتابعات البينية البسيطة (ISSRs) ، تم الحصول على ٤٥ ، ٣٨ و ٢٨ حزمة ( دن ا ) في كل من M0 ، M1 و M2 باستخدام خمس بادئات ISSR. حيث وجد ان اربع حزم ( دن ا ) كانت فريدة. وقد أعطت البادئة HB14 عدد ٢ حزمة دن ا فريدة ذات وزن جزئي ٨٩٥ و ١٠٥٠ زوج من القواعد وظهرتا في الجيل الاول M1 في الصنف جيزة ١١١ بعد المعاملة بجرعة ١٥٠ جراي ، بينما اعطت البادئة ٨٤٤ و البادئة ٨٦٢ حزمة واحدة فريدة ، حيث ظهرت في الصنف جيزة ١١١ العينة المرجعية في الجيل M0 وكانت لهما وزن جزئي ٨٨٨ و ٩٠٠ زوج من القواعد على الترتيب. وقد اعطت البادئة UBC-827 العدد الاكبر من حزم دن ا (٧) ، بينما لم تكبر أي حزم فريدة.