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Phenotypic and molecular polymorphism in M_2 generation of soybean plants derived through application of gamma irradiation

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The impact of (100 to 500 Gy) of γ-irradiation on M_2 plants of three cultivars of soybean and their association with ISSR fingerprinting have been studied. Morphological variation was scored as plant size, roots nodule formation, leaf shape, size and colour, pod shape, seed hilum colour, testa surface texture and testa colour. Ten ISSR primers successfully amplified 137 reliable markers. In general, reduction in polymorphic markers were associated with increased γ-irradiation doses for the three soybean cultivars. Pearson correlation coefficient indicated that the number of unique and polymorphic markers are generally correlated with the visible variations in some morphological traits which may be regarded as potential mutations. These findings indicate the importance of assessing changes in phenotypic traits, induced by mutagens, and their associated genetic markers.

Keywords: Gamma radiation, Soybean, ISSR fingerprinting, phenotypes.

INTRODUCTION

Mutation breeding through genetic variability has been and will be a viable approach for the development of crop cultivars with desired traits that can play a vital role to combat global food insecurity. Radiation has been the most frequently used method to create genetic variation by modifying DNA sequences randomly to increase genetic variability in new mutants for practical breeding application by generating new cultivars with better characteristics (Maluszynski et al. 1995). The induced mutants have been freely available for plant breeding and many of them have made impact on increasing yield and quality of several seed propagated crops (Ahloowalia et al. 2004). Such mutants have been utilized in mutation breeding as a simple, low cost and time saving method and a tool for improvement of certain qualitative and quantitative traits in many crop plants (Arefrad et al. 2012). According to the International Atomic Energy Agency (IAEA) report (2016), gamma rays has been the most employed methods to develop 64% of the radiation-induced mutant cultivars. It shortens the time taken for the development of cultivars via induced mutation compared to hybridizations and controlled crosses (Maluszynski et al. 2000).

In recent years, gamma (γ-) irradiation has been successfully used to induce genetic variation in grain legumes (Pavadai et al. 2010; Amri-Tiliouine et al. 2018). In mung bean, many phenotypic alternations have been associated with γ-irradiation, such as chlorophyll deficient and flower colour mutations and dwarfism, early flowering, large pod size (Kumar et al. 2009). Gamma-irradiated cowpea seeds at 196 to 245 Gy successfully generated useful agronomic traits capable of conferring advantages for increased yield, easy harvesting and insect tolerance, some mutants included had variegated and pigmented leaves, leaflets having tendrils, pods above canopy, dark and light green pods, wide angled and hairy pods, dwarf plants and early maturing plants (Adekola and Awoleye, 2007; Nurmansyah et al., 2018). In cowpea cv. Kaha1, seed coat colour, and seed eye pattern of M_2 black seeded mutant line were segregated in the M_3 plants ranging from cream to reddish brown and three eye patterns were distinguished from each other (Gaafar et al. 2016). Substantial morphological variability was detected among cowpea genotypes after mutagenesis across generations including flowering ability, flower maturity, and seed colours and grain yields (Horn et al. 2016).

Kumar et al. (2009) observed wide range of chlorophyll mutations (albina, chlorina, viridis and xantha) and morphological changes in M_2 mutants of mung bean cultivars following exposure to doses of 10 to 60 kR of γ-irradiation. The spectrum and frequency of chlorophyll mutations increased with γ-irradiation dose. Similarly, Bhosale and Hallale (2011) reported a range of chlorophyll and viable morphological mutations in M_2 generation of black gram (*Vingo mungo*) after exposure to γ-irradiation with doses of 10 to 50 KR; chlorophyll mutations were more frequent with γ-irradiation dose of 30 KR. Significant increase of growth rate, grain yields and yield components were observed in M_2 populations of different soybean cultivars at low doses of γ-irradiation. However, a significant reduction was observed for higher doses; the levels of changes varied among cultivars. Potential high yielding mutants were identified in progenies of irradiated seeds (Satpute and Fultambkar, 2012, Gaafar et al. 2017, El-Azab et al. 2018**)**.

The application of PCR based DNA markers for breeding and selection, has been a novel strategy and a powerful methodology for exploiting plant variability and has significant advantages compared with conventional breeding methods (Jiang, 2015). The random amplified polymorphic DNA (RAPD) amplified by arbitrary primers (Williams et al. 1990) and the inter-simple sequence repeats (ISSRs) which reveal regions that lie within the microsatellite repeats in the genome (Zietkiewicz et al. 1994; Reddy et al. 2002). Both were used to determine intra-genomic and inte-rgenomic diversity and has been commonly applied in legume diversity analysis and breeding (Alzate-Marin et al. 2020). Increased level of polymorphism was observed in progenies of cowpea plants exposed to 100 Gy and 200 Gy of γ-irradiation using ISSR and RAPD markers. Genetic variation among the M_2 genotypes of; var. Kaha 1 and var. Dokki 331 was more pronounced compared to other cultivars, as estimated by the cluster analysis of seed protein, RAPD and ISSR markers (Badr et al. 2014).

El-Gazzar et al. (2016) reported evident variation in ISSR profiling by seven primers in response to γ-irradiation doses in M_2 plants of six *faba* bean varieties. Different morphological mutants in M_2 population of *faba* bean were associated with unique markers. Molecular characterization using RAPD and ISSR markers following γ-irradiations for M_1 and M_2 cultivars of common beans show association of some fingerprints with cultivars differences and others were associated with γ-irradiations exposure (El-Lithy et al. 2016). Low doses of γ-irradiation (100 and 200 Gy) enhanced the mitotic activity in the root tip meristems of the three soybean cultivars (Crawford, Giza 35, and Giza 111) that has been reflected as increased vegetative growth and improved yield. However, high doses (300 to 600 Gy) reduced mitotic index, vegetative growth, and yield; the later dose was lethal to cv. Crawford (El-Azab et al. 2018).

The objectives of the present study are to investigate the phenotypic variations and molecular polymorphism revealed by ISSR fingerprinting in M_2 generations derived through application of γ-irradiation at different doses to explore genetic diversity in three different soybean cultivars; Giza 111, Giza 35, and Crawford. The induced morphological variations and ISSR markers were detected and correlated together in M_2 plants

MATERIALS AND METHODS

Plant materials

Three soybean cultivars; Giza 111, Giza 35, and Crawford were used in this study. Seeds were obtained from the Legumes and Field Crops Research Department, Agriculture Research Centre (ARC) in Giza, Egypt. 150-200 Dry seeds of each soybean cultivars were exposed to five doses of gamma irradiation at the National Centre for Radiation Research and Technology (NCRRT), Nuclear Research Centre, Inshas, Egypt using Co^{60} as a source. The applied doses were 100, 200, 300, 400 and 500 Gray (Gy); seeds of control samples were not exposed to irradiation. The seeds were planted in 10 pots replica, each pot contains 15 seeds. The number of plants were lighted to 10 plants/pot 20 days post germination. Then the plants allowed to complete maturation and M_1 seeds formation. hundred M_1 seeds from each treatment and from controls were planted in 10 pots, allowed to grow for M_2 plants. Dark and wrinkled testa were induced in 500 Gy irradiated M_1 seeds of cv. Crawford that failed to survive or germinate to M_2 plants. So, all analysis for Crawford cultivar were performed for only 100, 200, 300 and 400 Gy of γ-irradiation doses.

Recording morphological variations

Morphological observations were carefully and continuously recorded for M_2 plants throughout vegetative and reproductive growth stages. The following traits were recorded using Nikon COOLPIX L820 digital camera after eight weeks of germination; root nodule, leaf shape, size and colour, pod shape, hilum colour, testa surface texture and testa colour. Plant size was recorded four weeks after germination. Pod and seed traits were recorded at harvesting.

DNA extraction and ISSR fingerprinting

Total genomic DNA was isolated from green leaves according to the method of Vejlupkova and Fowler's (2003). ISSR amplification was performed using 14 primers and Mytaq Red DNA polymerase master mix (BIOLINE cat # BIO-21108) according to manufacture instructions. Briefly, the reaction mix was composed of 1X PCR red master mix buffer, 2.0 µl of 15 pm/µl of each corresponding primer, 1.0 µl of DNA template (~30 ng), 0.125µl of MyTaq™ DNA polymerase (5U/µl), then the total volume was adjusted to 25 µl using sterile distilled water. The amplification reactions were performed in Thermal Cycler (Biometra, Germany) as follow: 1st cycle of 4 min at 95°C for initial denaturation, followed by 39 cycles of 60 sec at 95°C (denaturation), 45 sec at (each primer corresponddding annealing temperature, table 1), 2 min at 72°C (extension). A final extension was carried out for 10 min at 72°C, the reactions were held at 4°C.

The PCR products were resolved in 1.2% agarose gel and visualized using UV-transilluminator (Virballurmate-Germany). A digital image of the ethidium bromide-stained gel was captured using Nikon COOLPIX L820 digital camera and the bands (ISSR markers) were scored using Quantity one Software version 4.6.2.70. The bands occurrence was scored as binary data; 1 for presence and 0 for absence. The number of unique bands, polymorphic bands, monomorphic bands, and the percent of polymorphism for each primer and each treatment were calculated. A 1kb+ GeneRuler DNA ladder (Thermo scientific #SM 1331) was used to determine the size of the ISSR fragments.

Data analysis

Pearson correlation coefficient values (r values) between each morphological mutation trait and the number of unique or polymorphic ISSR markers were calculated using regression parameter of the data analysis package in Microsoft EXCEL version 2010. Genetic similarity using the Neighbour joining (NJ) method among the M_2 genotypes induced following γ-irradiation of parents' seeds with the applied doses, were calculated using PAST Version 3.24 (Hammer, 2001) at https:// folk.uio.no/ohammer/past/.

RESULTS

Potential morphological mutations induced by γ-irradiation treatments

The low doses of 100 Gy and 200 Gy showed increased plant size of four weeks old plants of the three used soybean cultivars. On contrast, a reduction in plants size was associated with the high doses of 400 Gy and 500 Gy (Fig 1a). Lower number of root nodules was observed in eight weeks old Crawford roots at 300 Gy of γ-irradiated in comparison to those of the control plants and plants from seeds exposed to 100 Gy and 200 Gy of γ-irradiation (Fig 1b). Leaf shape, size and colour mutations were clearly observed; elliptic leaves were more common in 100 Gy and 200 Gy γ-irradiated plants in comparison to their controls with lanceolate leaves. On contrast, high doses of 500 Gy negatively affects leaf size and colour. Xantho-chlorophyll mutation was observed as yellowish elliptical small leaves in cv. Giza 35 plants grown from parent seeds exposed to 500 Gy of γ-irradiation (Fig 1c)

Yield components associated phenotypic traits include changes in pod shape, seed colour and texture. Curved pods were observed in M_2 plants of Giza 111 and Giza 35 grown from seeds exposed to 500 Gy (Fig 2a). White hilum colour was induced after parental seeds exposure to the low dose of 200 Gy of **γ-**irradiation compared to black hilum of control plants in all cultivars (Fig 2b).

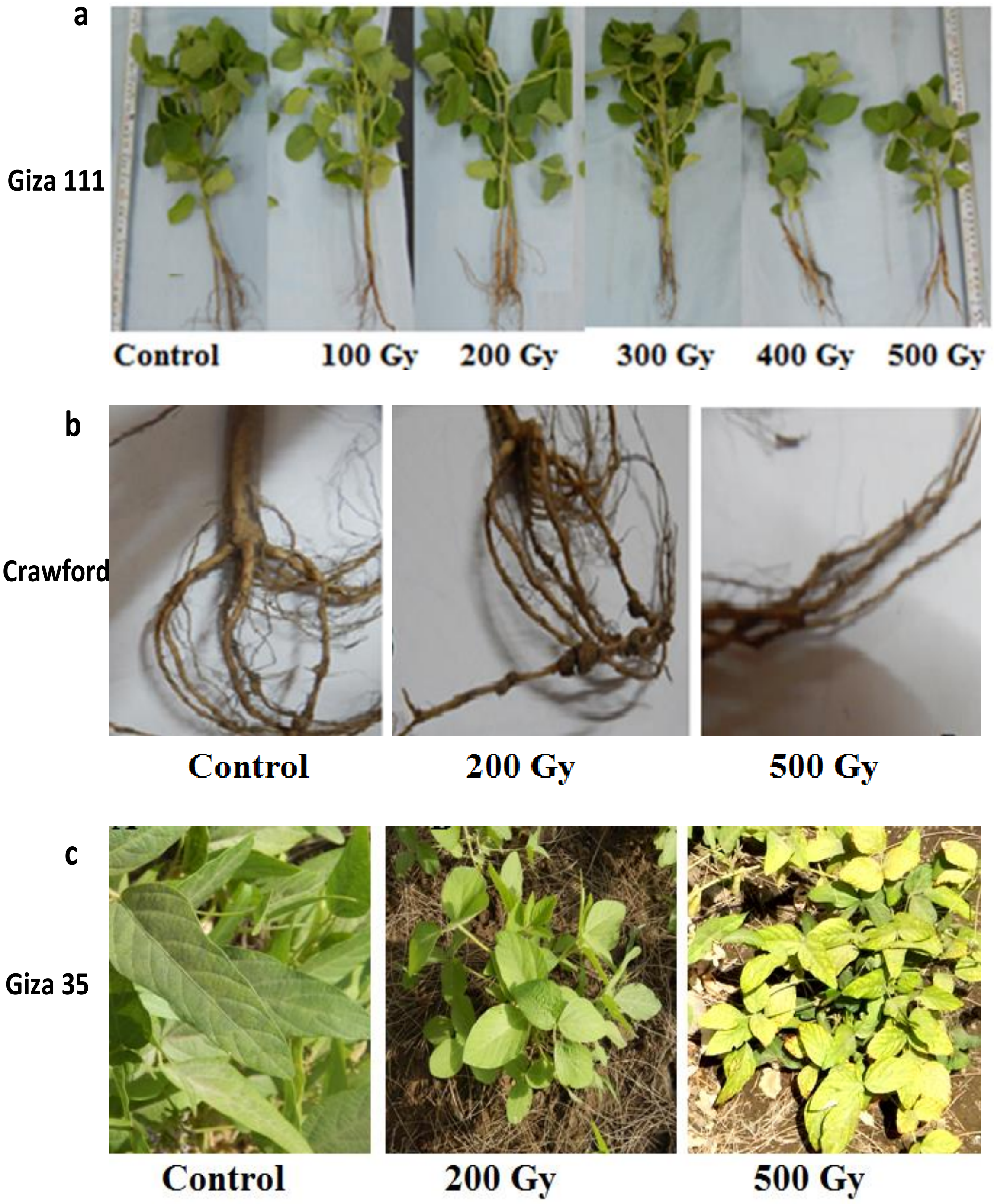

Figure 1: Photographs illustrating examples of changes in vegetative parameters: a) changes in plant size of M_2 Giza 111 plants at the age of four weeks after exposure of their parental seeds to 100, 200, 300, 400 and 500 Gy of γ-irradiations in comparison to their control. b) the ability of nodule formation in M_2 Crawford plant roots at the age of eight weeks after exposure of their parental seeds to 200 and 500 Gy of γ-irradiations in comparison to their control. c) changes in leaf shape, size and colour in Giza 35; green lanceolate leaf in control, large green elliptic leaf at 200 Gy of γ-irradiation, small and yellow elliptic leaf at 500 Gy of γ-irradiation.

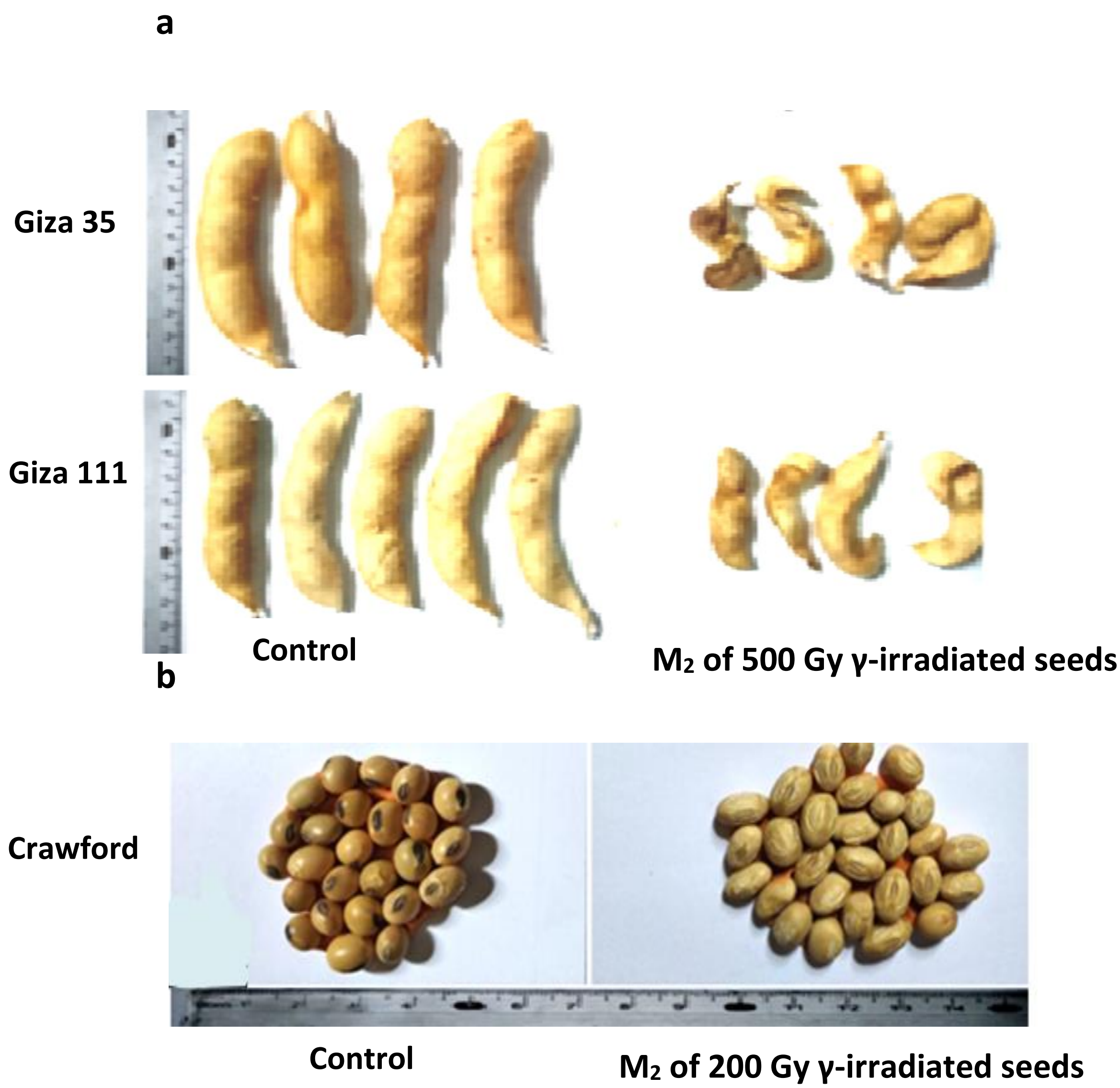

Figure 2: Photographs illustrating examples of pod shape and seeds phenotypes of M_2 Giza 111, Giza 35 and Crawford cultivars after exposure of their parental seeds to different doses of γ-irradiations in comparison to their control. a) straight normal pods of M_2 Giza 35 and Giza 111 control plants in comparison to curved deformed pods at 500 Gy of γ-irradiation. b) black hilum in smooth control seeds while slightly wrinkled seeds and white hilum at γ-dose 200 Gy of Crawford.

ISSR polymorphism for M_2 γ-irradiated plants

Fourteen ISSR primers were used to investigate genetic variability induced by γ-irradiation in the three soybean cultivars. Only ten primers successfully amplified 137 reliable mar-kers; 47 were monomorphic, 67 were polymorphic and 23 were unique (Table 1, Fig 3).

The amplified markers ranged in size between 5053 bp and 217 bp with total polymorphism 65.7%. The highest percentage of polymorphism (80%) was scored for primer UBC-812 whereas the lowest percentage (50.0%) was scored for the primer UBC-861. A maximum number of 21 markers were amplified by primer HB-10 while only seven markers were amplified by primer UBC-844. Noticeable reduction in polymorphic markers with increased γ-irradiation doses was perceived in the three studied soybean cultivars (Fig 4).

The dendrogram based on ISSR markers showing the genetic similarity of M_2 soybean genotypes; the control plants of cv Giza 111, cv. Giza 35 and cv Crawford and their variants produced by their parental seeds exposure to 100, 200, 300, 400 and 500 Gy of γ-irradiation (Fig 5). The cluster analysis generated two main groups, one contains the control plants and the genotypes produced by the low doses of 100 and 200 Gy γ-irradiation plants of both cv. Giza 35 and cv. Crawford (G1). While the second group is differentiated as two subgroups; one comprises the control plants and the genotypes produced by low doses of 100, 200 Gy and also the 300 Gy γ-irradiation of cv. Giza 111 (G2A) and the other subgroup includes the high doses of 300, 400 and 500 Gy of γ-irradiation of the three cultivars (G2B).

Pearson correlation of potential phenotypes and ISSR markers.

Pearson coefficient was calculated to find association between nine phenotypic variations in response to γ-irradiation and the ISSR polymorphic and unique markers (Table 2). Significant positive correlation was found between the number of unique bands and leaf shape (0.561) and hilum colour (0.487). On the other hand, a significant positive correlation was found between the number of polymorphic markers and most of the changed phenotypic traits. Plant size, leaf size and root nodules formation, all by value of (0.689), leaf shape (0.534) and leaf colour (0.549). The visible variations in morphological traits may be regarded as potential mutations induced by increased γ-irradiation. No correlations were found between the molecular markers and pod shape, testa surface and testa colour.

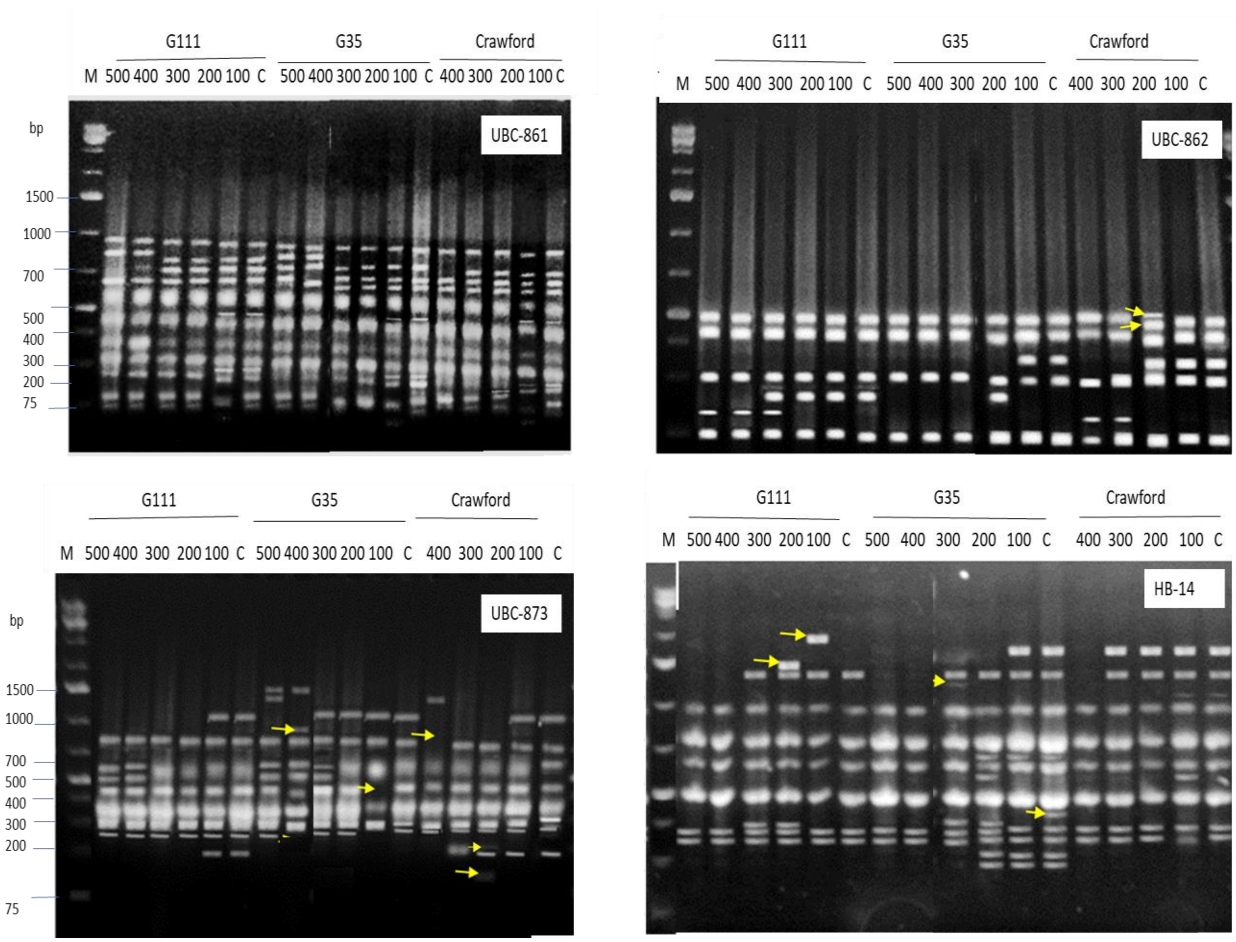

Figure 3: Examples of ISSR profiles produced in M_2 Giza 111, Giza 35 and Crawford cultivars after exposure of their parental seeds to 100, 200, 300, 400 and 500 Gy of γ-irradiations in comparison to their controls. M: 1kb+ DNA ladder. Arrows refer to the unique bands.

Table 1: List of selected ISSR primers and their codes, sequences, annealing temperatures (Tm), the number of amplified markers and percent of polymorphism for each primer in the M_2 genotypes of soybean. (Bold font; refers to primers that didn't produce bands).

Primer code	Tm °C	Primer sequence	Number of marker types			Total markers	Poly-morphism %
			Unique	Mono-morphic	Poly-morphic		
UBC-889	**60**	**(AGT)(CGT)(AGT)ACACACACACACAC**	-	-	-	-	-
UBC-885	**60**	**(CGT)(ACT)(CGT)GAGAGAGAGAGAGA**	-	-	-	-	-
UBC-891	**60**	**(ACT)(ACG)(ACT)TGTGTGTGTGTGTG**	-	-	-	-	-
UBC-868	**40**	**GAAGAAGAAGAAGAA**	-	-	-	-	-
UBC-812	54	GAGGAGGAGGAGGAGAT	2	3	10	15	80.00
UBC-827	52	ACACACACACACACACG	2	6	8	16	62.50
UBC-842	58	GAGAGAGAGAGAGAGACTG	2	3	4	9	66.66
UBC-844	56	CTCTCTCTCTCTCTCTGC`	2	2	3	7	71.43
UBC-861	50	ACCACCACCACCACC	0	8	8	16	50.00
UBC-862	60	AGCAGCAGCAGCAGCAGC	2	3	4	9	55.56
UBC-873	48	GACAGACAGACAGACA	5	3	4	12	75.00
HB-14	44	GTGTGTGTGTGTCC	4	6	8	18	66.70
HB-10	44	GAGAGAGAGAGACC	2	8	11	21	61.90
ISSR5	50	ACGACGACGACGGAC	2	5	7	14	64.29
		Total number of markers	23	47	67	137	65.70

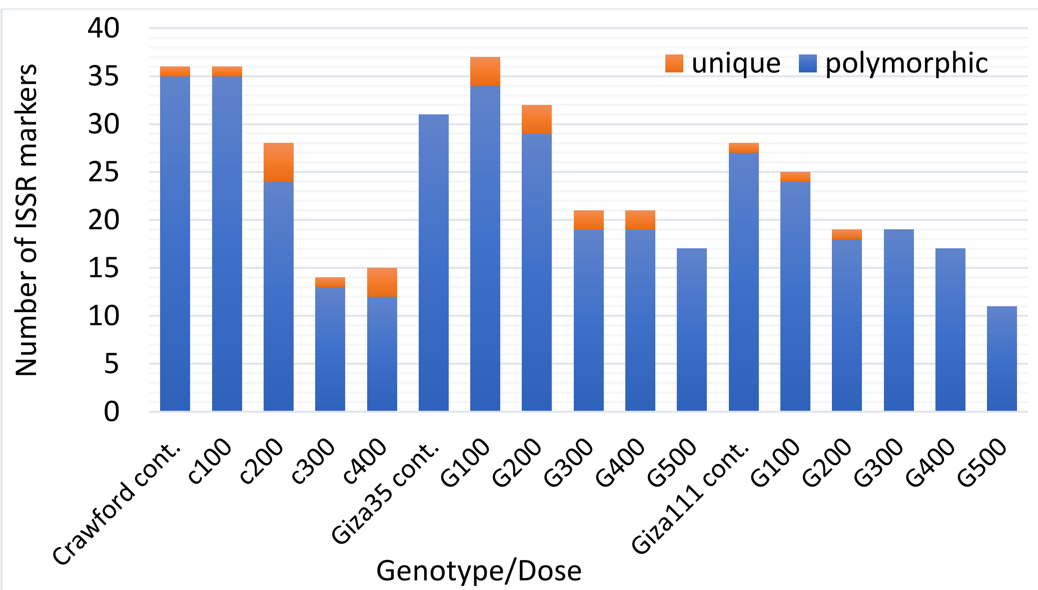

Figure 4: Number of total amplified polymorphic and unique markers in M_2 Giza 111, Giza 35 and Crawford cultivars after exposure of their parental seeds to 100, 200, 300, 400 and 500 Gy of γ-irradiations in comparison to their controls.

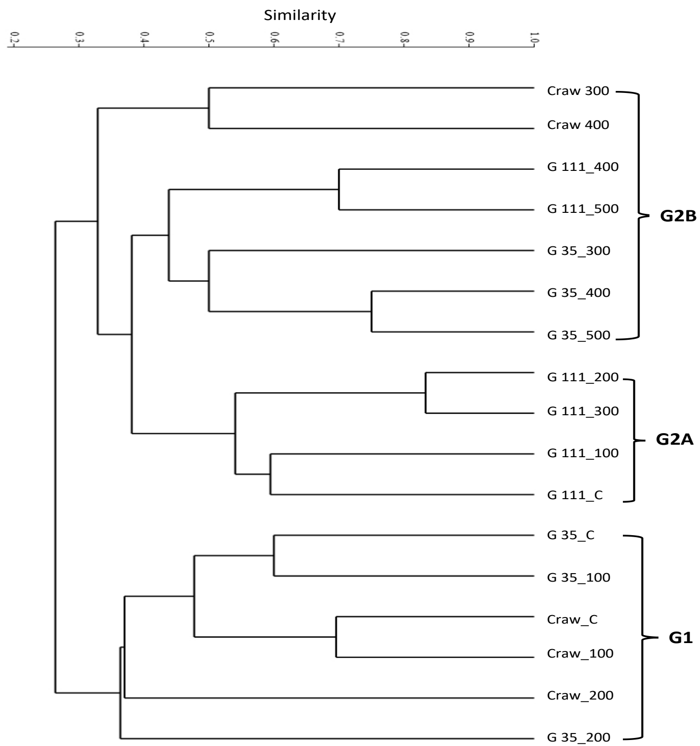

Figure5: Dendrogram based on the ISSR markers showing the genetic similarities between M_2 Giza 111 (G 111), Giza 35 (G 35) and Crawford (Craw) cultivars after exposure of their parental seeds to 100, 200, 300, 400 and 500 Gy of γ-irradiations in comparison to their controls.

Table 2: Pearson correlation coefficient (r- value) between the nine morphological phenotypes obtained and the unique or polymorphic ISSR markers, the significance was calculated as P value.

Phenotypic traits	Unique markers		Polymorphic markers	
	r-values	P values	r-values	p values
Plant size	0.408	0.104	0.689*	0.002
Nodule formation	0.408	0.104	0.689*	0.002
Leaf shape	0.561*	0.019	0.534*	0.027
Leaf size	0.408	0.104	0.689*	0.002
Leaf colour	0.132	0.612	0.549*	0.022
Pod shape	0.400	0.111	0.402	0.110
Hilum colour	0.487*	0.047	0.449	0.070
Testa surface	0.400	0.111	0.402	0.110
Testa colour	0.400	0.111	0.402	0.110
	* Significance at P values ≤ 0.05			

DISCUSSION

The increased plant size of four weeks old plants of the three used soybean cultivars at low doses of 100 Gy and 200 Gy is congruent with reports by El Azab et al. (2018) that the low doses of γ-irradiation accelerated cell cycle, increased the mitotic activity in root meristems and increased cell numbers and consequently enhanced plant growth expressed as root and shoot size. Fehér et al. (2008) has attributed the acceleration of cell cycle to the involvement of reactive oxygen species in the cell cycle activation at the G0-to-G1 transition stage in plant cells.

In the current study, a spectrum of morphological mutations was induced by different applied γ-irradiation doses. In accordance with Khan et al. (2000) who showed a wide range of morphological mutations in chickpea that was induced by a separate and simultaneous application of γ-irradiation with GA3. These morphological mutations affecting plant size, growth habit, branching, stem structure, foliage colour, leaf type, pod and seed type, the frequency of these mutants increased with γ-irradiation dose (Khan et al. 2000). Similar morphological mutations were also reported in horse gram with γ-irradiation (Priyanka et al. 2019) and *Vicia faba* plants (El-Gazzar et al. 2016).

It is worth noting that the chlorophyll mutations were associated with alternations in leaf size. The higher doses of 500 Gy induced xantho-chlorophyll mutation with small elliptical leaves. This in agreement with Soehendi et al. (2007) who stated that the modification obtained by γ-irradiation of mung bean (*Vigna radiata* L.) leaflet size, type and colour could affect leaf canopy and alter seed yield. Suggesting the presence of two independent loci for controlling leaflet number; N1 and N2. In which the loci containing N2 allele expresses pleiotropic effect on both leaflet number and leaflet size. An additional locus with S and s alleles controls leaflet size and S is tightly linked with N2 locus. These possibilities were regarded as useful source for understanding the genetic control of leaflet and regulation of their size, shape, and form. Bhosale and Hallale (2011) reported adverse leaf mutation in M_2 of γ-irradiated black gram plants including chlorophyll mutations of albino, coppery, light green, and variegated leaf colours. Various leaf shape and composition mutations such as waxy leaf, lanceolate, narrow rugose leaf, round cuneate leaf, unifoliate, and tetra-foliate leaf were observed at different γ-irradiation dose but was more pronounced in response to 30 kR.

γ-irradiation interact with cellular molecules to produce free radicals in the cells. These radicals can modify important components of plant cells (Reisz et al., 2014) and induce mutations which create variability in quantitatively and qualitatively inherited traits (Muduli and Misra, 2007). Chlorophyll and leaf mutations also could be attributed to the altered expression levels of some genes associated with chlorophyll biosynthesis or chloroplast and leaf development (Ryu et al. 2018 and Yeboah et al. 2018).

Yield associated mutations for pod and seed traits were observed. The severity of this kind of mutation were linked to high doses. M_2 plants of 500 Gy γ-irradiation showed curved pods with fewer seeds number in all cultivars compared to straight pods in their controls. Furthermore, sterility of dark and wrinkled testa of M_1 seeds of cv. Crawford were observed at the same dose that failed to germinate to M_2 plants compared to smooth and yellow testa in non-irradiated seeds. Several pod mutations were recorded in faba bean following gamma irradiation such as lobed pods associated with fewer seeds per pod and partial sterility and undeveloped seeds (El-Gazzar, 2016). Mutational changes include seed-coat colour and seed eye pattern were induced by different doses of γ-irradiation. White hilum colour was dominant to the low dose of 200 Gy seeds rather than the black one of the control seeds of the three soybean cultivars. Similar mutations were recorded in cowpea (Badr et al. 2014) and *Phaseolus vulgaris* (Armelim et al. 2006 and El-Lithy et al. 2016) that was cultivar and dose dependent. These mutational effects could be induced due to changes in the cellular structure and metabolism, like dilation of thylakoid membranes, alteration in photosynthesis and pigment accumulation which may later change the colour and texture of leaves and seeds (Gudkov et al. 2019).

Various phenotypic mutations were associated with increased number of polymorphic ISSR markers at low doses of 100 and 200 Gy in contrast to lower number of markers generated at high doses of 300, 400 and 500 Gy in the three soybean cultivars. These results agree with the reports by Mudibu et al. (2011) and Badr et al. (2014) who scored an increase in polymorphism in the M_2 plants of cowpea after γ-irradiation of their parental seeds. Molecular polymorphism also agrees with the results of Gaafar et al. (2017) who used ISSR markers to detect genetic responses of the soybean cultivars to γ-irradiation. The changes in morphological traits were accompanied with a marked modulation in the DNA profile. Variation in ISSR markers profiling in responses to γ-irradiation induce genetic diversity in soybean that was associated with increased quantitative morphological traits (Wang et al. 2012). The ISSR polymorphism might be connected to structural rearrangements in DNA caused by different types of DNA damages (breaks, transpositions, deletion, etc.) stimulated by gamma irradiation (Kim et al. 2019). γ-irradiation induced genetic variability, may be attributed to various mutated regions of the genome (Donà et al. 2013). These regions are of interest in plant breeding using molecular fingerprinting. The rate of mutation across the genome is not uniform and differs significantly due to the distribution of radiation sensitive regions across the whole genome. This leading to unequal distribution of mutations in the plant genome in response to irradiation. Also, the ability of the plant to repair these genome damage in subsequent plant generations is variable. (Van der Vyver et al. 2011)

The cluster analysis of the ISSR fingerprinting discriminates the M_2 genotypes into categories indicating that the applied doses of γ-irradiation generated inherited genetic variation that was superior to the natural variation among the three used cultivars of soybean i.e. Crawford, Giza 35 and Giza 111. The first major group contains the control plants of cv. Crawford and cv. Giza 35 and the genotypes produced by the low doses of 100 and 200 Gy γ-irradiation of both cultivars. While the second group is subdivided in to two clades. The control plants and the genotypes of cv. Giza 111 produced by the low doses of 100 and 200 Gy of γ-irradiation in a clade. The other clade includes genotypes produced by the high doses of 300, 400 and 500 Gy of γ-irradiation of the three cultivars. Interestingly, within the major group, the genotypes of each cultivar were separated in independent subgroups. Badr et al. (2014) showed that in cowpea, the cluster analysis of molecular data revealed that the grouping of the cultivars was more dependent on the natural variation between them rather than the radiation dose used to generate variations. It worth noting that, the maximum dose used for cowpea was 200 Gy. However, in the current study we applied up to 500 Gy of γ-irradiation.

Pearson coefficient indicated a significant positive correlation between most phenotypic traits observed and polymorphic ISSR markers such as plant size, root nodules formation and leaf traits. A significant correlation was detected between leaf shape and hilum colour with the unique markers. These correlations do not identify genes concerned with specific traits (Ryu et al. 2018) but express DNA damage response associated with changes in phenotypic traits that may be visualized as different ISSR fingerprinting particularly as polymorphic and unique markers.

CONCLUSION

In conclusion, some γ-irradiation doses effectively induced morphological mutations in plant size, leaf traits as well as pod shape and some seed traits. Although these potential mutations do not have much economic importance, they are useful in identifying the effective dose that would increase the genetic variability. These mutants may be useful in the subsequent generations. The ISSR fingerprinting provided a discriminative power in detecting polymorphism and could be significantly correlated with the different phenotypic variations.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

ERS Supervised the laboratory experiments and participated in the data collection, analyses and manuscript writing. EME performleed the experimental and field research and conducted data analyses, MSAS supervised the practical work and revised the manuscript. AB proposed the research topic, supervised the research work, revised the article preparation and writing. All authors read and approved the final version.

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