RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2019.45.436

ScienceAsia 45 (2019): **436–445**

Physiological and molecular studies on ISSR in two wheat cultivars after exposing to gamma radiation

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Received 17 Feb 2019
Accepted 8 Aug 2019

ABSTRACT: Grains of two bread wheat cultivars (*Triticum aestivum*), Sids-1 and Sakha-93, were irradiated with gamma rays at dose levels (0.0, 100, 200, and 300 Gy) to study the effects of gamma irradiation on physiological characteristics and genetic variation of wheat. Irradiation dose level of 200 Gy increased chlorophyll a, b, and total chlorophyll contents significantly in both cultivars. Also, proline content increased with increasing irradiation dose level, the highest concentrations were recorded at 300 Gy for Sids-1 and Sakha-93 cultivars as compared to the control. Inter-Simple Sequence Repeat (ISSR) markers have been done to help understanding their genetic differences. Eight ISSR primers (14A, 44B, HB-08, HB-10, HB-11, HB-12, HB-13, and HB-15) exhibited polymorphism with the un-irradiated and irradiated two wheat cultivars. These pimers successfully showed different banding patterns with several amplicons varied from 4 for (14A) to 15 for (HB-10). These 72 amplicons for the two cultivars and an average of 9.0 amplicons with mean of 67.96% polymorphism and resolving power (Rp) of 3.41. It is also cleared that radiation is more effective on Sids-1 cultivar with 55.5% polymorphism than on Sakha-93 cultivar with 51.9% polymorphism. Total amplicons found in Sids-1cultivar were 64, ten of them were unique amplicons (UA): 5 UA(+) and 5 UA(−). Irradiation dose (200 Gy) showed the highest number of UA (3 UA− and 3 UA+) in Sids-1cultivar. While, total amplicons found in Sakha-93 cultivar were 58, eight of them were 5 UA(+) and 3 UA(−). Irradiation dose (300 Gy) showed the highest number of UA as 4 UA+. It could be concluded that gamma irradiation of wheat grains produced an appropriate number of generated variations and that ISSR analysis given a useful molecular marker for the symmetry of the mutants.

KEYWORDS: wheat, inter simple sequence repeat ISSR markers, gamma radiation

INTRODUCTION

Wheat (*Triticum aestivum*) is an important staple crop around the world, its importance has increased due to experienced food storages and its role in world trade. Wheat occupied the first place among the cereal crops, ranging 30% of all cereal food in the world and main food for over one-third of world people that supplies about 20% of the total food calories directly or indirectly for humankind. To meet higher demands by increasing population, wheat production must be increased[1]. Previously, morphological, cytogenetic, pedigree or chemical analysis were used to investigate plant diversity[2]. Estimation of genetic diversity using molecular markers is a basis for realizing genomic structure, the characterization and maintenance of genetic variation in plant germplasm, identifying genes underlying important traits and applying optimal breeding strategies for crop improvement[3]. Using markers and realization of polymorphic nucleotide sequences dispersed throughout the genome have given new facilities for evaluation, diversity and detecting of inter and intra-species genetic relationships[4]. Several molecular markers are available for investigation of genetic diversity. The ISSR marker overcomes most of these molecular markers, where, they have advantage of relatively low cost, high polymorphism and good reproducibility. It is considered as a new method of molecular markers, is based on inter tandem repeats of short DNA sequences. These inter repeats are highly polymorphic in their sizes even among closely related genotypes as the result of the deficiency of evolutionary functional constraints in these functioning regions. Also, it is a technique that is widely used for assessing the alteration in DNA sequences induced by mutagenic agents such as gamma rays[5]. Additionally, ISSRs have been used successfully in genome mapping for a variety of crop species including maize, rice, barley and wheat[6,7]. Gamma irradiation is considered as physical mutagen imposing considerable effects on

physiological and biochemical processes in plants[8]. Using of gamma irradiation technique represents a significant role in plant breeding programs and genetic studies are aimed to improve yield and produce desirable traits in many crops under both normal and stress conditions[9]. Also, it may cause genetic variability that enable plant breeders to select new genotypes by improving characteristics such as precocity, tolerance to stresses, grain yield and quality[10]. The mutants developed in wheat had great potential for direct release and to include them in cross breeding program[11]. Many studies showed that the relative doses of ionizing irradiation could be useful for acceleration of cell proliferation, germination rate, cell growth, enzyme activity, stress resistance, and crop yields[12]. The biological influence of gamma rays is due to the interaction with atoms or molecules in the cell, especially water to generate new radicals[13]. These radicals can damage or modify important components of plant cells and have been reported to affect differentially the morphology, anatomy, biochemistry and physiology of plants depending on the irradiation dose level[14]. Furthermore, irradiation by gamma rays leads to increase the level of DNA break formation that can be mitigated through direct identification of genotypes with DNA based assays[15].

The aim of this work was to determine the effect of gamma irradiation on some physiological characteristics as well as genetic variability detected with ISSR markers. Also, the genetic diversity and relationships between Sids-1 and Sakha-93 wheat cultivars.

MATERIALS AND METHODS

Plant materials

Grain of two bread wheat cultivars (*Triticum aestivum*), Sids-1 and Sakha-93, used in the present study were obtained from Agriculture Research Centre, Ministry of Agriculture, Giza, Egypt. The two cultivars were irradiated with gamma rays at dose levels (0.0, 100, 200, and 300 Gy with a dose rate of 1.9 kGy/h). The source of irradiation was installed at the National Centre for Radiation Research and technology, Atomic Energy Authority, Nasr City, Cairo, Egypt. Irradiated and un-irradiated grains were sown at the experimental farm belonging to the Natural Products Department, to obtain M_1 of the grains. Soil mechanical and chemical analysis was performed[14].

Four plants per replicate were randomly chosen for determining photosynthetic pigments and proline content.

Determination of photosynthetic pigments

Chlorophyll a, b and carotenoids were determined in wheat leaves by the spectrophotometric method[16]. Fresh samples (0.5 g) were homogenized in a mortar with 85% acetone in the presence of washed dried sand and a little amount of $CaCO_3$ ($\approx$ 0.1 g) to neutralize organic acids in the homogenate. The homogenate was then filtered through sintered glass funnel. The residue was washed several times with acetone until the filtrate became colourless. The combined extract was completed to a known volume. The optical density of obtained extract was determined at 662, 644 nm for chlorophyll a and chlorophyll b, respectively, and 440.5 nm for carotenoids. The pigment contents were calculated as mg/g fresh weight. The concentration of chlorophyll a, b and carotenoids were calculated by the formula (in mg/l):

$$\text{Chlorophyll a} = 9.784 \times A_{662} - 0.99 \times A_{644},$$
$$\text{Chlorophyll b} = 21.426 \times A_{644} - 4.65 \times A_{662},$$
$$\text{Carotenoid} = 4.695 \times A_{440.5} - 0.268 \times c(\text{a}+\text{b}),$$

where c(a+b) is the sum of chlorophyll a and b in mg/l. The results were calculated as mg/g fresh weight.

Determination of proline content

Proline content was determined by the method of Bates[17]. In brief, 100 mg of frozen plant materials were homogenized in 1.5 ml of 3% sulphosalicylic acid and the residue was removed by centrifugation. Two ml glacial acetic acid and 2 ml acid ninhydrin reagent (1.25 g ninhydrin warmed in 30 ml glacial acetic acid and 20 ml 6 M phosphoric acid until dissolved) were added to 100 µl of the extract for 1 h at 100 °C and the reaction was then completed in an ice bath. One ml of toluene was added to the mixture, then warmed to room temperature and its optical density was measured at 520 nm. The amount of proline was determined from a standard curve in the range of 20–100 µg.The results were expressed as mg/g of proline equivalent of the fresh weight of the samples.

Genomic DNA isolation for ISSR analysis

Total genomic DNA was isolated and purified from frozen M_1 leaves of the un-irradiated and irradiated two wheat cultivars using DNeasy plant Mini Kit (QIAGEN, Chatsworth, CA). The concentration of DNA was determined at a wavelength of 260/280 nm and

Table 1 List of primers (ID) and their nucleotide sequences used in the PCR analysis for ISSR procedure.

No.	ID	sequence
1	14A	5′-CTC TCT CTC TCT CTC TTG -3′
2	44B	5′-CTC TCT CTC TCT CTC TGC -3′
3	HB-08	5′-GAG AGA GAG AGA GG -3′
4	HB-10	5′-GAG AGA GAG AGA CC -3′
5	HB-11	5′-GTG TGT GTG TGT TGT CC -3′
6	HB-12	5′-CAC CACCAC GC -3′
7	HB-13	5′-GAG GAGGAG GC -3′
8	HB-15	5′-GTG GTGGTG GC -3′

the quality was verified by electrophoresis on 1.4% agarose gel.

ISSR-PCR analysis: polymerase chain reaction

ISSR-PCR reactions were performed using twenty random primers, amplification was conducted in 25 µl reaction volume containing the following reagents: 2.5 µl each of dNTPs (2.5 mM), $MgCl_2$ (2.5 mM), and 10 × buffer, 3.0 µl of primer (10 pmol), 3.0 µl of template DNA (25 mg/µl), 1 µl of Taq polymerase (1 U/µl), and 12.5 µl of sterile H_2O. The PCRs (Techni TC-512 PCR System) were programmed for one cycle at 94 °C for 4 min. followed by 45 cycles of 1 min at 94 °C, 1 min at 57 °C, and 2 min at 72 °C the reaction was finally stored at 72 °C for 10 min. The PCR products were separated on 2% agarose gels and fragment sizes were estimated with the 100 bpDNA ladder. Only eight from 20 primers succeeded to generate reproducible polymorphic PCR products. Table 1 lists the base sequences of these DNA primers that produced informative polymorphic amplicons.

Statistical analysis

A complete randomized block design with three replicates was used. The data were presented as the mean ± SD. All the statistical analyses were performed using an ANOVA, and Duncan's multiple range tests[18] was applied to compare the results of the experiments ($p \leqslant 0.05$).

Molecular analysis

DNA banding pattern of each primer were analysed by GelAnalyser3 software which scoring clear amplicons as present (1) or absent (0) for each primer in binary data matrix. From this matrix, resolving power (Rp) of each primer was calculated according to Prevost and Wilkinson[19] using the formula:

$$\mathrm{Rp} = \sum I_b, \quad I_b = 1 - (2 \times |0.5 - p|),$$

where, I_b (amplicon in formativeness) was calculated for each amplicon scored individually by the primer, p being the ratio of studied lines containing the I amplicon.

Also, cluster analysis and similarity index were performed from binary data using agglomerative hierarchical clustering derived from unweighted pair-group average UPGMA method by XLSTAT.7 computational software.

RESULTS AND DISCUSSION

Chlorophyll content

The estimation of important biochemical characteristics such as chlorophyll a, b and carotenoids contents in the two Egyptian wheat cultivars (Sids-1 and Sakha-93) are presented in Fig. 1 and Fig. 2, respectively. Photosynthesis is one of the most studied processes under the effects of gamma irradiation, a gradual increase in chlorophyll a, b, total chlorophyll, and carotenoids contents have been observed in the present study, which reached the maximum content at the dose level of 200 Gy (1.067, 0.6239, 1.6909, and 0.333 mg/g FW), respectively, for Sids-1 cultivar and (1.344, 0.6092, 1.9532, and 0.2231 mg/g FW), respectively, for Sakha-93 cultivar. Then all these contents decreased slightly at the dose level of 300 Gy (0.8569, 0.555, 1.4119, and 0.1602 mg/g FW), respectively, for Sids-1 cultivar and (0.9487, 0.5533, 1.502, and 0.1497 mg/g FW), respectively, for Sakha-93 cultivar. In the same concern, chlorophyll a, b, and total chlorophyll levels significantly increased in wheat with increasing gamma irradiation dose levels until 200 Gy[20]. In view of this, photosynthetic pigments can be highly decreased by irradiation high dose levels, with concomitant loss of photosynthetic capacity[21]. According to transmission electron microscope observations, chloroplasts were highly sensitive to gamma radiation compared to other cell organelles, especially thylakoids being heavy swollen[9]. Likewise, a high dose of gamma rays up to 500 Gy reduced chlorophyll content by 80.9% and reduced the organized structure of grana and stroma thylakoid[22].

Proline content

The results in Fig. 3 showed the effect of different doses of gamma irradiation on the proline content in the two wheat cultivars (Sids-1 and Sakha-93). We found that the content of proline increased by increasing gamma irradiation dose level. Increase in proline content can be helpful in maintaining

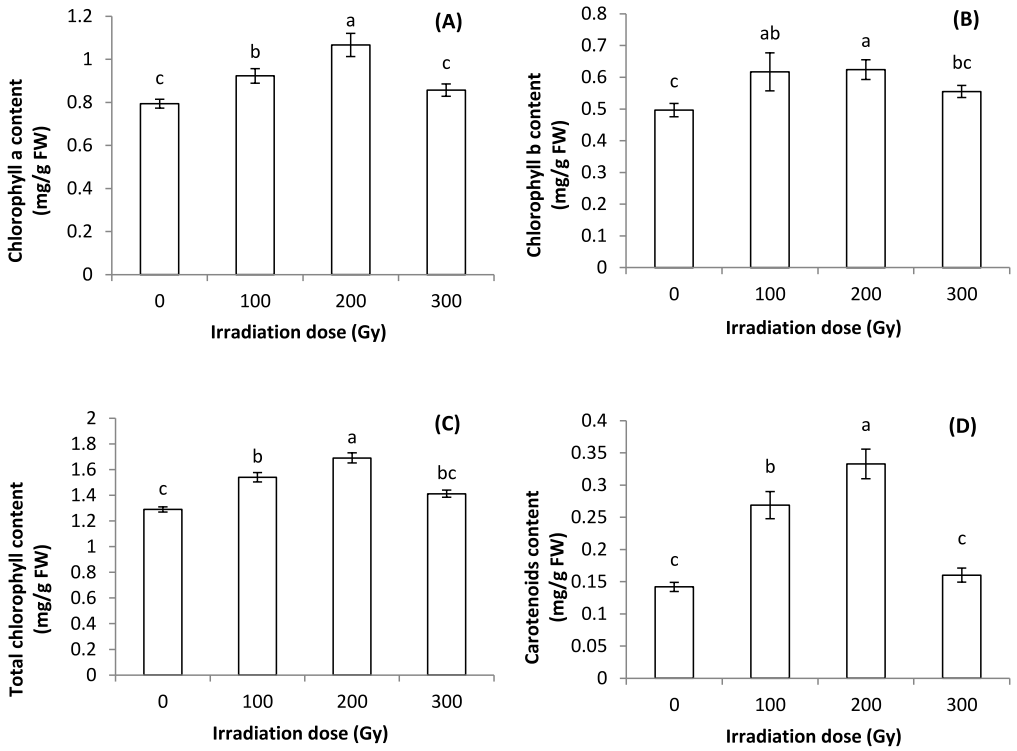

Fig. 1 Effect of gamma irradiation on chlorophyll a (A), chlorophyll b (B), total chlorophyll (C), and carotenoid (D) contents (mg/g FW) of Sids-1 Egyptian wheat cultivar. Vertical bars show SD (n = 3) and different letters indicate statistically significant differences at $p \leqslant 0.05$.

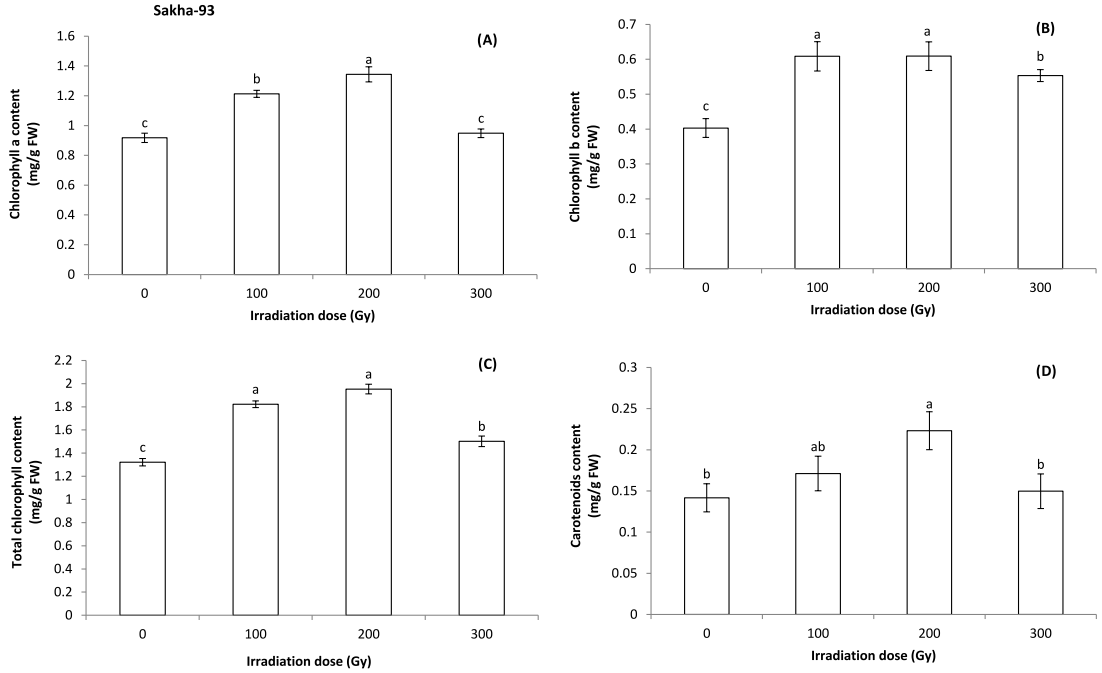

Fig. 2 Effect of gamma irradiation on chlorophyll a (A), chlorophyll b (B), total chlorophyll (C), and carotenoid (D) contents (mg/g FW) of Sakha-93 Egyptian wheat cultivar. Vertical bars show SD (n = 3) and different letters indicate statistically significant differences at $p \leqslant 0.05$.

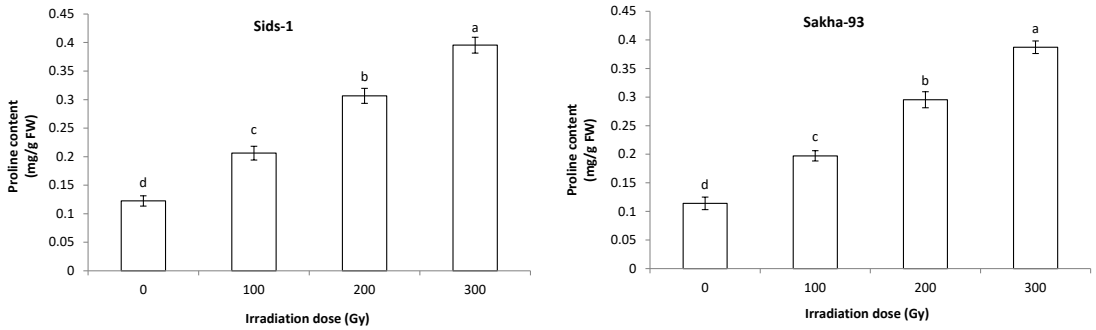

Fig. 3 Effect of gamma irradiation on proline content (mg/g FW) of Sids-1 and Sakha-93 Egyptian wheat cultivars. Vertical bars show SD (n = 3) and different letters indicate statistically significant differences at $p \leqslant 0.05$.

osmoticum under various environmental stresses. In the present study, the highest concentrations (0.3954 and 0.3870 mg/g FW) were recorded at 300 Gy for Sids-1 and Sakha-93 cultivars, compared to the control (0.1225 and 0.1142 mg/g FW) for Sids-1 and Sakha-93 cultivars, respectively. Increase proline content with increasing irradiation dose level in the present study confirms the role of proline as a compatible solute. These results are in agreements with Akshatha et al[23] who reported that the proline content increased with increasing irradiation doses in *Terminalia arjuna* Roxb. Also, Aly et al[14] reported that exposure to gamma rays significantly increased proline accumulation in wheat leaves especially at dose level 300 Gy in M_2. Ionizing radiation enhanced proline content referring that proline has an important role in the defence systems against gamma rays[22]. Radiation stimulates formation of reactive oxygen species (ROS), which is extremely toxic to plant cells. Proline serves as scavenger of ROS and can maintain the structure and function of macromolecules such as DNA, protein and membranes[24]. The magic effect may be due to increase proline accumulation in the plant cells. Also, the hydrophilicity of proline and other compatible solutes play great role to placed water molecules around nucleic acid, proteins and membranes during drought period. Hence the interaction of proline and altered proteins causes increase in the stability of proteins[25]. When plants are subjected to water stress they increase their content of proline, this enables to improve the capacity of the cell to maintain its turgor pressure at low water potential. This appears to be essential for physiological processes such as photosynthesis, enzyme activity and cell expansion[26].

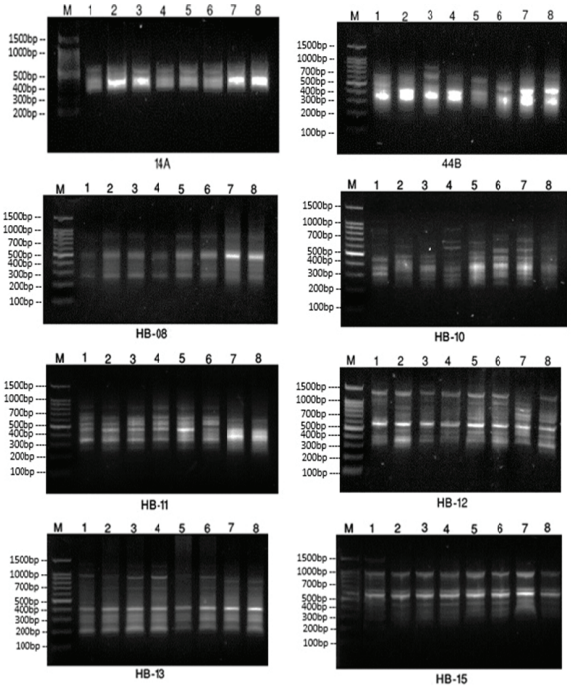

Fig. 4 Agarose gel electrophoresis of ISSR amplifications of the Egyptian two wheat cultivars Sids-1 and Sakha-93 with primers (14A, 44B, HB-08, HB-10, HB-11, HB-12, HB-13, and HB-15) lane M, DNA marker (100–1500 bp); lane 1 Sids-1 0.0 Gy, lane 2 Sids-1 100 Gy, lane 3 Sids-1 200 Gy, lane 4 Sids-1 300 Gy, lane 5 Sakha-93 0.0 Gy, lane 6 Sakh-93 100 Gy, lane 7 Sakha-93 200 Gy, and lane 8 Sakha-93 300 Gy.

ISSR banding patterns

The ISSR technique has been done in this study for M_1 (un-irradiated and irradiated samples to see the induced molecular markers changes), eight ISSR primers (14A, 44B, HB-08, HB-10, HB-11, HB-

Table 2 Total number of amplicons, polymorphic amplicons, unique amplicons and percentage of polymorphism obtained from the irradiated and un-irradiated Sids-1 and Sakha-93 wheat cultivars using ISSR.

Primer name	Molecular size range (bp)	TA	Poly.%	Rp	Sids-1							Sakha-93						
					TA	A(−)			A(+)			Poly.%	TA	A(−)			A(+)			Poly.%
						PA (−)	UA(−)		PA (+)	UA(+)				PA (−)	UA(−)		PA (+)	UA(+)		
							No.	MS,t		No.	MS,t				No.	MS,t		No.	MS,t	
14A	487–703	4	50.0	1.25	4	1	0	–	1	0	–	50.0	3	1	0	–	0	0	–	33.3
44B	333–802	6	100.0	3.50	6	3	2	402,200 556,200	2	2	620,200 802,200	83.3	5	1	1	556,100	3	0	–	100.0
HB-08	260–839	6	66.7	2.25	5	0	0	–	3	0	–	60.0	6	1	0	–	1	0	–	50.0
HB-10	250–1233	15	93.3	7.25	11	6	2	307,100 578,200	3	1	650,300	81.8	12	5	1	250,300	2	1	720,100	75.0
HB-11	282–981	10	70.0	2.75	10	2	0	–	2	1	425,200	40.0	7	2	2	282,300 558,300	0	0	–	57.1
HB-12	301–1824	11	63.6	5.25	9	4	1	1824,300	0	0	–	44.4	11	2	1	1054,300	1	1	441,200	45.5
HB-13	217–967	10	50.0	3.25	9	1	0	–	3	1	540,300	44.4	8	0	0	–	3	0	–	37.5
HB-15	327–625	10	50.0	1.75	10	4	0	–	0	0	–	40.0	6	0	0	–	0	1	327,100	16.7
Total	217–1824	72			64	16	5		9	5			58	12	5		10	3		
						21 (32.8%)			14 (21.9%)					17 (29.3%)			13 (22.4%)			
Main	–	9	67.96	3.41	8	2.625			1.75			55.5	7.3	2.125			1.625			51.9

TA: total amplicons in each cultivar; A(−): amplicons, appear in control and absent in one or more of the treatments; PA(−): polymorphic A(−); UA(−): unique negative amplicon, absent in one treatment; A(+): amplicons, absent in control and appear in one or more of the treatments; PA(+): polymorphic A(+); UA(+): unique positive amplicon, appear in one treatment; MS: molecular size (bp) for unique amplicon; t: control or treatments (Gy); Poly. %: polymorphism percent.

12, HB-13, and HB-15) exhibited polymorphism with the un-irradiated and irradiated two wheat cultivars, Sids-1, and Sakha-93 (Fig. 4 and Table 2). These primers detected total amplification fragments of 72 bands for the two cultivars and at an average of 9.0 bands with mean of polymorphic percentage of 68% and resolving power (Rp) 3.41. In addition, it is clear from Table 2 that the eight primers out of 20 succeeded in detecting large number of amplicons A(+) and A(−) which differ among irradiation treatments and their control of each cultivar. For wheat cultivar Sids-1 and its treatment, all primers successfully showed multiple band profiles (Fig. 4) with several amplicons varied from 4 (for 14A) to 11 (for HB-10), Table 2. In total, 64 amplicons were found, 35 (54.7%) of them were polymorphic; of which 10 (15.6%) were unique amplicons (UA), 5 UA(+) and 5 UA(−) in Sids-1 cultivar. In Sakha-93, total 58 amplicons were found, 30 (51.7%) of them were polymorphic; of which 8 (13.8%) were unique amplicons, 5 UA(+) and 3 UA(−). The highest number of UA was produced by the primer 44B while the primers 14A, HB-08, and HB-15 showed no appearance of UA amplicons. Furthermore, for wheat cultivar Sakha-93 and its treatments, all primers were successfully showed multiple band profiles, with number of amplicons varied from 3 (for 14A) to 12 (for HB-10) (Table 2). In total 58 amplicons, 51.9% of them were polymorphic. Eight out of 30 amplicons were UA (5 negative and 3 positive UA).

Additionally, from data of A(−) and A(+) which refer to the effect of treatments on the susceptibility of studied wheat cultivars for compatibility with ISSR primers, these results showed that the percentage of A(−) was greater in Sids-1 (A−% = 32.8) than in Sakha-93 (A−% = 29.3) while, A(+) was greater in Sakha-93 (A+% = 22.4) than in Sids-1 (A+% = 21.9). It could be concluded that the 200 Gy treatment of Sids-1 produced the greatest number of individual molecular markers (+3 and −3 of total of 10), otherwise the 300 Gy treatment of Sakha-93 cultivar resulted in the highest number of the individual molecular markers (+5 and −3 of total of 8). The best primer in the ability for illustrating the variation induced by gamma irradiation in both cultivars was HB-10. Sids-1 cultivar was most affected by gamma irradiation resulted in variation rate of 55.5% while only 51.9% was found in Sakha-93 cultivar.

The improvement in each one of the studied two cultivars and in between them could be associated with molecular markers as described in Table 2. There were 5 UA(+) and 5 UA(−) induced in Sids-1, three of them induced by the treatment 200 Gy. Otherwise, Sakha-93 performed eight amplicons 5 UA(+) and 3 UA(−), the irradiation dose level 300 Gy was the most effected dose induced four positive amplicons. These amplicons are in accordance with the physiological traits (chlorophyll and proline contents) as the most effective dose for chlorophylls was 200 Gy and 300 Gy for proline in the two cultivars.

The ISSR marker was an efficient and reproducible method to assess genetic diversity in wheat (*Triticum aestivum*), and could be used as highly informative markers for genome mapping and gene tagging because the evolutionary rate of change within microsatellites is considerably higher than many other types of DNA markers. This agreed with, Abd El-Aziz et al[27] who demonstrated that gamma rays were succeeded for inducing desirable changes in okra at the two phenotypic and molecular levels. Also, Singh and Datta[28], who reported that gamma irradiation encouraged phenotypic changes in wheat, whereas total leaf mass, plant mass and the tiller number increased three times more than control as a result of gamma irradiation. Gamma radiation at dose levels 50, 100, 200, and 300 Gy induced more genetic variation in the genotypes of var. Kaha 1 and var. Dokki 331 Cowpea compared to other varieties, as estimated by the cluster analysis of seed protein, RAPD and ISSR markers[29]. In the same concern Žiarovská et al[30] indicated that the different ISSR fingerprint patterns of the amaranth mutant lines when compared to the Ficha cultivar and K-433 hybrid, ISSR specific profiles may be part of a consequence of the complex response of the intergenic space of mutant lines to the gamma-radiance. Genetic analysis using ISSR of the different populations of Chinese fir in Fujian province exhibited a greater level of genetic diversity than that generated from the populations in Taiwan[31]. Polylocus ISSR-PCR markers can be used for characterizing gene pools and for molecular genetics identification of populations and breeds, including starlet populations and replacement brood stocks[32]. Disappearance of ISSR bands in some M_1 and M_2 plants could be referred to damages of DNA like single or double-strand breaks, modified bases, oxidized bases, and bulky adducts. In addition, DNA-protein crosses links, point mutations and rearrangement of chromosomes were induced by gamma irradiation[29]. Furthermore, it has been shown that free radicals interact with biomolecules as DNA and remove electrons from them, so they

damage both structure and activity of the DNA. During ISSR amplification, as Taq polymerase reaches DNA damage, there could be a blockage by potential dismantling of the enzyme-DNA, which will show the loss of ISSR bands. While, the appearance of new bands may be because of the effect of mutation rather than of DNA damage[33]. Treating plants with highest doses of gamma rays resulted in the highest reduction in content of genomic DNA. Similarly, the appearance or disappearance of bands under gamma irradiation might be considered as molecular markers for radiation processes[34]. 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[35]. Heiba et al[36] demonstrated that, RAPD, ISSR, and SSR markers play vital and successful role to differentiate between all the genotypes used concerning salt stress in wheat which could be helpful in the enhancement of cereals production in Egypt. Also, cited that this technology can be used as an indicator of molecular breeding in wheat, to increase ability of abiotic stress tolerance of the studied lines and using it in local breeding program. Expressions of candidate genes in several major metabolic pathways and stress responses were positively correlated with heat tolerance manifested by the genetic variations in leaf chlorophyll content, photochemical efficiency, and membrane stability in fine fescue cultivars[37]. Elshafei et al[38] identified several types of molecular markers associated with the three physiological traits in wheat under water-stress and identified five markers for each physiological linked to leaf chlorophyll content, flag leaf senescence and cell membrane stability traits, respectively, as indicator for drought tolerance gene in wheat. These markers might be used for marker-assisted selection. Iqbal et al[39] showed that the wheat genotype association with the levels of proline during induced drought stress and the relationship between pattern of drought responsive biochemical attributes and DNA markers in the selected wheat genotypes was recognized to select drought tolerant genotypes. In the same concern, Aly et al[40] demonstrated that peroxidase and polyphenol oxidase activity and gene expressions can be used as biochemical and molecular markers to detect the resistance or susceptibility nature of wheat cultivars against salinity in integration with gamma irradiation.

Cluster analysis as revealed by ISSR

The genetic variations among the control (un-irradiated) and irradiated two wheat cultivars Sids-1 and Sakha-93 were determined by Jaccard's genetic similarity coefficients, ranged from 0.614 and 0.916 with an overall mean of 0.825 and 0.875 for Sids-1 and Sakha-93, respectively, depending on ISSR marker. The UPGMA cluster analysis of Jaccard's similarity coefficients produced a dendrogram (Fig. 5) which showed the genetic relationship among the un-irradiated and irradiated samples for both cultivars. The analysis illustrated that γ-irradiated for the two cultivars and the control fell

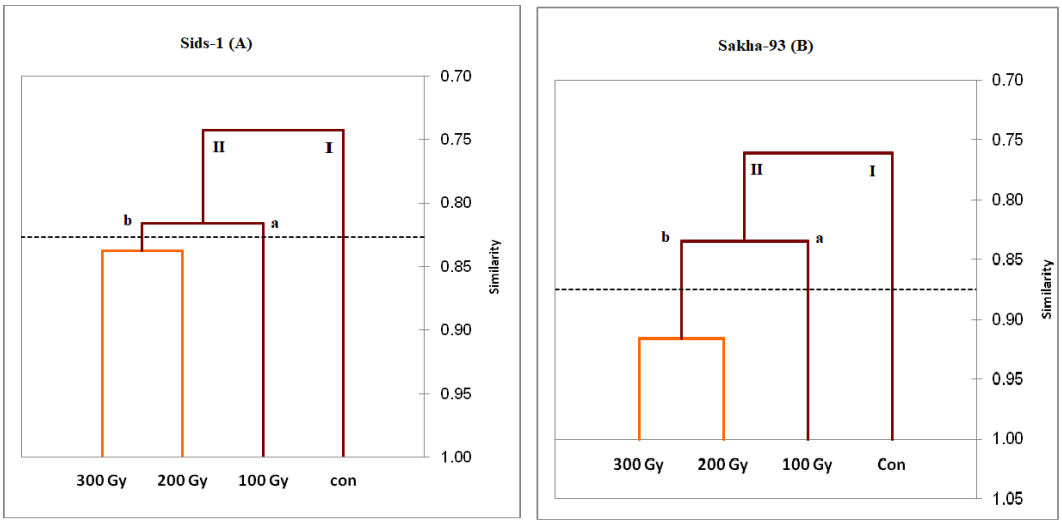

Fig. 5 Dendrograms for the irradiated and un-irradiated two Egyptian wheat cultivars Sids-1 (A); (Con, 100, 200, and 300 Gy) and Sakha-93 (B); (Con, 100, 200, and 300 Gy) based on similarity coefficients of ISSR data. Con = control.

into two main clusters. The first main cluster *I* composed of the control, while the second cluster II involved two groups: the first group (a): comprised of 100 Gy. The second group (b) involved the doses 200 and 300 Gy. Irradiation treatments applied in this study were succeeded in inducing genetic variations among molecular and phenotypic levels of the two wheat cultivars. It was found that the most genetic distance was more pronounced in Sids-1 than in Sakha-93. These cluster analyses, showed true variation among control and irradiation treatments for both cultivars. This variation can be useful in wheat breeding programs by selection. This agreed with, Singh and Datta[28], who demonstrated that gamma irradiation, encouraged phenotypic changes in wheat. The molecular analysis of groundnut using ISSR markers has shown that gamma ray irradiation at a dose of 100 Gy increased the level of genetic variability[41]. The difference between the highest and the lowest values of genetic distance showed a wide range of variability among the accessions evaluated[42].

It could be summarized that using these advanced molecular techniques, new genes can be potentially identified by the plant breeders as the result of in vitro mutagenesis treatments. The results showed changes in non-irradiated and irradiated treatments, based on inter microsatellite length polymorphism. Hence it can be concluded that gamma ray treatment was an effective way for mutation be induction in wheat and the mutants could successfully identified through ISSR analysis. Hence the recent progress in mutation breeding studies in relation with new technologies is quite important to contribute new advancement to plant breeding programs.

Acknowledgements: Authors would like to thank the Atomic Energy Authority for their support and Ministry of Scientific Research for funding this study through the frame of Tunisia-Egypt cooperation project, fourth call, cod No. 14/4/30.

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