International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 26-11-2022, Accepted: 13-12-2022, Published: 29-12-2022
Volume 7, Issue 12, 2022, Page No. 174-180

Genetic diversity in adults of *Earias insulana* (Boisd.) resulted from gamma irradiated pupae

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Abstract
The goal of the current study was to evaluate the molecular diversity in adults of the spiny bollworm *Earias insulana* that irradiated with doses of 25, 50, 100, and 200Gy as full-grown pupae. Data from ISSR-PCR showed that the DNA bands of the groups under study varied. According to the similarity index, the control adult pupae had a low similarity index to the other irradiated adults (as full grown larvae). In this investigation, six primers of uninformed sequences were tested to check genomic DNA of the *E. insulana* adults. The molecular weight varied between 145 bp to 1435 bp. In the current search, irradiated adults were contrasted to unirradiated ones to explore any potential changes to the DNA structure that might be a result of gamma radiation treatments. The obtained results showed polymorphic, monomorphic and unique bands in the fingerprints generated in adults resulted from gamma irradiated full grown when compared with normal adults. Moreover, the highest numbers of amplified bands were 8 generated from primer HB13, whereas the lowest numbers of amplified fragments were 3 detected from two primers 49B and HB8 in gamma irradiated adults as pupae. The resulted ISSR-PCR patterns using primer HB10 revealed the lowest value of similarity index (0.33) between irradiated adults "as full grown pupae" with 50Gy and the unirradiated adults which displays the greatest degree of alteration in DNA sequence and arrangement.

Keywords: spiny bollworm, gamma radiation, DNA alteration, PCR, polymorphism

Introduction
Cotton occupies a vital role in agrarian economy of Egypt. It provides raw material to domestic and the other subsidiary industries employs millions of hands and ears substantial amount of foreign exchange. Cotton plants with its green leaves, many large open flowers, nectarines on every leaf, flowers and large number of fruits seems special attract for the insect pests under natural conditions [1]. In Egypt and around the world, the spiny bollworm (SBW), *Earias insulana* (Boisd.), is one of the most devastating pests of cotton, maize, and okra [2]. The larva feeds on the plant's fruiting portions in cotton cultivates and may kill one cotton boll out of every two or three plants [3]. *Earias* spp. is a destructive that decreases about 50% of cotton quality bout 40% of the yield [3]. To control *Earias* spp. infestations, a variety of pesticides from various classes that contain pyrethroids, organophosphates, and carbamates are used[4]. The wide use of chemical insecticides created problems such as developed of resistance in the pest, resurgence of secondary pests, environmental pollution and health hazards.
In order to categorise pesticide resistant populations and design effective control tactics, the expansion of molecular markers provides valuable information on the genetic diversity of the insect [5]. The polymerase chain reaction (PCR) is a helpful tool with several uses in daily life. It has been possible to distinguish between distinct animal species and their resistance strains using random amplified polymorphic DNA. This method is often used to distinguish between populations within a species. A single primer with any nucleotide sequence can be used to detect nucleotide sequence polymorphisms using this rapid and simple method [6]. An inter simple sequence repeat (ISSR) primer made from dinucleotide or trinucleotide simple sequence repeats enables the detection of polymorphisms in inter-microsatellite loci.
Because gamma rays have a shorter wavelength, they have more energy per photon than X-rays. At the moment, the primary sources of gamma rays used in radiobiological activity are cobalt-60 and cesium-137. At the level of medicinal plants studies, radiation is a good inducer for many useful improvements. Good results had been obtained at the level of germination, yield growth parameters and that it enhanced active ingredients [7-9].
Therefore in the current study, biological aspects as well as molecular variations could be evaluated in the adults resulted from pupae irradiated with four doses of gamma radiation compared with unirradiated adults.

Materials and Methods
1. Insect rearing
The original colony of spiny bollworm, *Earias insulana* (Boisd.) was collected from okra fields, Qaha District, Qalubia Governorate at late season of 2002 and reared in Bollworms Research Department, Plant Protection Research Institute, Agricultural Research Center (ARC), Dokki, Giza. The insects were reared under laboratory conditions of 25± 2ºC temperature; and 60±5% relative humidity on okra pods.

2. Irradiation Technique
Gamma Cell-40 (Cobalt Co^{60} Irradiation Unit) at the National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Egypt, was used to irradiate full-grown pupae with different doses of gamma radiation (25, 50, 100, and 200Gy). The cell's dosage rate was 0.815KGy/h.

3. DNA isolation and PCR condition for ISSR

The bulked DNA extraction was performed using D Neasy insect Mini Kit (QIAGEN). PCR was performed in 30-µl volume tubes according to Williams *et al*. [6]. The DNA amplifications were performed in an automated thermal cycle (model Techno 512) programmed for one cycle at 94ºC for 4 min followed by 45 cycles of 1 min at 94ºC, 1 min at 57ºC, and 2 min at 37ºC, the reaction was finally stored at 72ºC for 10 min. 15 ISSR primers were tested and we observed fragments of 14 sequences only (table 1). The amplified ISSR products were separate by electrophoresis on 2% agarose gel with 0.5x TBE buffer. After staining with ethidium bromide, banding patterns were visualized with a UV transilluminator.

Table 1: List of the primer names and their nucleotide sequences used in the study for ISSR procedure

Primer Name	Sequences
49A	5` CAC ACA CAC ACA AG 3`
49B	5` CAC ACA CAC ACA GG 3`
HB-8	5´ GAG AGA GAG AGA GG 3`
HB-10	5` GAG AGA GAG AGA CC 3`
HB-11	5` GTG TGT GTG TGT TGT CC 3`
HB-13	5` GAG GAG GAG GC 3`

4. Data analysis

UVP-England Program, a complex piece of software from Gel Works ID, was used to create the similarity matrices.

Similarity index

In order to compare trends within and between populations, the similarity index was utilised. This index measures how widely bands are shared and is created as follows:

$$2N_{ab} / (N_a + N_b)$$

Where $\mathbf{N_{ab}}$ is number of bands common to individuals a, b. N_a and N_b are total number of bands in a and b, respectively. The value produced by this index ranges from zero, respecting no bands sharing, to 1, respecting complete identity [10].

Results

Inter simple sequence repeat (ISSR)-PCR pattern profiles were created by using primers on DNA from the *Earias insulana* adults produced from gamma irradiated full grown pupae with 25, 50, 100 and 200 Gy as well as unirradiated ones. In this investigation, six primers of arbitrary sequences as shown in Table (1) were used to screen pooled genomic DNA of the *E. insulana* field population adults. The total number of produced fragments were 32 distributed as 6, 3, 3, 5, 7 and 8 generated by primers 49A, 49B, HB8, HB10, HB11 and HB13; respectively. The molecular weight alternated from 145 bp to 1435 bp. The utmost and least molecular sizes were identified by using HB13 primer. In the present experiments, adults who had received radiation as full-grown pupae were matched to controls to examine any potential DNA structural changes brought on by gamma radiation treatments.

The obtained results showed the fingerprints generated in adults resulted from gamma irradiated full grown pupae by each primers, 49A, 49B, HB8, HB-10, HB11 and HB13 revealed polymorphic, monomorphic and unique, profiles for the pest subjected to gamma radiation doses, as well as the untreated one. These primers gave good amplification with distinct fragments.

Using primer 49 A revealed some variability between the efficiency of different gamma irradiated doses under study on genetic variation of the *E. insulana* adults resulted from pupae exposed to varying levels of gamma radiation. As noticed in (Table 2) and illustrated in Figure (1), the DNA pattern created by ISSR-PCR amplification of the adult homogenates using primer 49A confined a total of 6 bands. There were 6 bands in the untreated larvae compared with 4,6, 3 and 6 fragments in irradiated adults with 25, 50, 100 and 200Gy; respectively. Amplified fragment of 825 bp was shared in control and adults irradiated with 25, 50 and 200 Gy. Two fragments of 615 and 470 bp were shared between control and gamma irradiated adults with 50 and 200 Gy. Three common fragments of 415, 300 and 200 pb were detected in control and all irradiated adults with different doses used. Using primer 49A, the PCR patterns produced were able to distinguish between the control and the four dosages of gamma radiation given to the tested pest. In the tissues of the studied pest's related adults, the fingerprints made by primer 49A exhibited three polymorphic and three monomorphic profiles.

Table 2: Total number and size of ISSR-PCR fragments created by primer 49A in *Earias insulana* adults resulted from pupae irradiated with different doses of gamma radiation.

MW-bp	MW	Control	25Gy	50Gy	100Gy	200Gy	Polymorphism
Band1	825	825	825	825	.....	825	Polymorphic
Band2	615	615	.....	615	....	615	Polymorphic
Band3	470	470	.....	470	.-----	470	Polymorphic
Band4	415	415	415	415	415	415	Monomorphic
Band5	300	300	300	300	300	300	Monomorphic
Band6	200	200	200	200	200	200	Monomorphic
Total	6	6	4	6	3	6	……

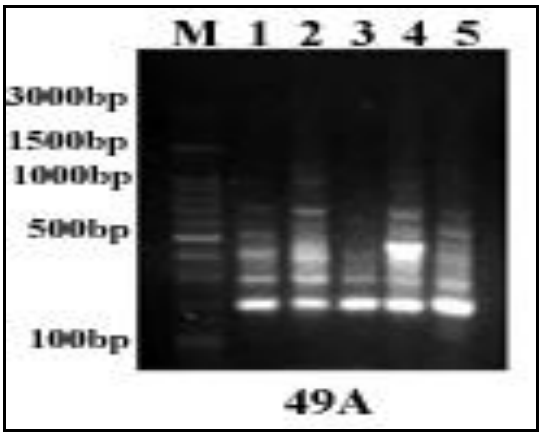

Fig 1: ISSR-PCR produced for gamma irradiated *Earias insulana* adults compared with control using primer 49A.

lane 1=Control, lane 2 =25 Gy, lane 3=50 Gy, lane 4= 100 Gy and lane 5= 200 Gy.

The similarity index test indicated a main drop from 1.00 to 0.67. Considering this, highly genomic structure could be observed in adults resulted from gamma irradiated pupae treated with 100 and 200 Gy where the similarity index equal 0.67 (Table3). The highest similarity index value was noticed between control compared with 50 and 200 Gy as well as between 50 and 200Gy where the similarity index recorded 1.0

Table 3: Estimated similarity index between *Earias insulana* adults resulted from pupae irradiated with different doses of gamma radiation using primer 49A.

Treatments	Control	25 Gy	50 Gy	100 Gy	200 Gy
Control	-----	0.80	1.00	0.67	1.00
25 Gy	-----	-----	0.80	0.86	0.80
50 Gy	-----	-----	-----	0.67	1.00
100 Gy	-----	-----	-----	-----	0.67
200 Gy	-----	-----	-----	-----	-----

Using primer 49 B revealed some variability between the efficiency of different gamma irradiated doses under study on genetic variation of the *E. insulana* adults resulted from pupae irradiated with different doses of gamma radiation. As noticed in (Table 4) and illustrated graphically in Figure (2), the ISSR-PCR pattern formed by amplification of the adult homogenates DNA using primer 49B confined a total of 3 bands. Two common amplified fragments of 375, 3000 bp were detected in all irradiated adults as well as the control. Only one amplified fragment of 200 bp was detected only in the adults irradiated with 25, 50 and 200 Gy of gamma radiation and absent in both control and adult irradiated to 100 Gy. The fingerprints produced by primer 49B exposed two monomorphic and 1 polymorphic fragment.

Table 4: Total number and size of ISSR-PCR fragments created by primer 49B in *Earias insulana* adults resulted from pupae irradiated with different doses of gamma radiation:

MW-bp	MW	Control	25Gy	50Gy	100Gy	200Gy	Polymorphism
Band1	375	375	375	375	375	375	Monomorphic
Band2	300	300	300	300	300	300	Monomorphic
Band3	200	……..	200	200	.-----	200	Polymorphic
Total	3	2	3	3	2	3	……

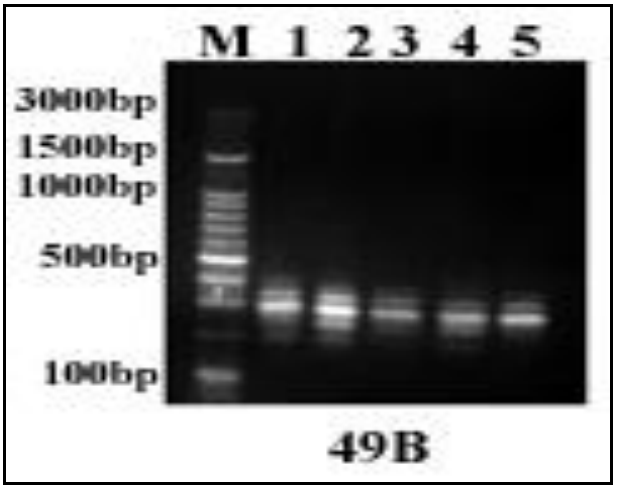

Fig 2: ISSR-PCR produced for gamma irradiated *Earias insulana* adults as pupae compared with control using primer 49B.

lane 1=Control, lane 2 =25 Gy, lane 3=50 Gy, lane 4= 100 Gy and lane 5= 200 Gy.

The data of similarity index values presented in Table (5) and depicted in figure (2) demonstrated that the control as well as gamma irradiated adults were similar to each other approximately, where the similarity index values alternated between 0.80 and 1.00.

Table 5: Estimated similarity index between *Earias Insulana* adults resulted from pupae irradiated with different doses of gamma radiation using primer 49B:

Treatments	Control	25 Gy	50 Gy	100 Gy	200 Gy
Control	-----	0.80	0.80	1.00	0.80
25 Gy	-----	-----	1.00	0.80	1.00
50 Gy	-----	-----	-----	0.80	1.00
100 Gy	-----	-----	-----	-----	0.80
200 Gy	-----	-----	-----	-----	-----

The ISSR-PCR analysis of the extracted DNA samples using primer HB8 revealed some variability between the efficiency of different gamma irradiated doses under study on genetic variation of the *E. insulana* adults resulted from irradiated pupae. As presented in (Table 6) and illustrated graphically in Figure (3), there were two to three generated bands of DNA fragments that distinguish between the control and the four tested dosages of gamma radiation. One common amplified band of 3000 bp was detected in all irradiated adults as well as the control. Only one amplified fragment of 485 bp was detected only in the control and adults irradiated with 50 and 200 Gy of gamma radiation and absent in the adults gamma irradiated adults irradiated with 25 and 100 Gy. Only one amplified band of 365 bp was appeared in control and adults exposed to 25, 50 and 200Gy of gamma radiation. The fingerprints generated by primerHB8 revealed one monomorphic and two polymorphic profiles.

Table 6: Total number and size of ISSR-PCR fragments created by primer HB8 in *Earias insulana* adults resulted from pupae irradiated with different doses of gamma radiation:

MW-bp	MW	Control	25Gy	50Gy	100Gy	200Gy	Polymorphism
Band1	485	485	…….	485	……..	485	polymorphic
Band2	365	365	365	365	……	365	polymorphic
Band3	300	300	300	300	.300	300	monomorphic
Total	3	3	2	3	1	3	……

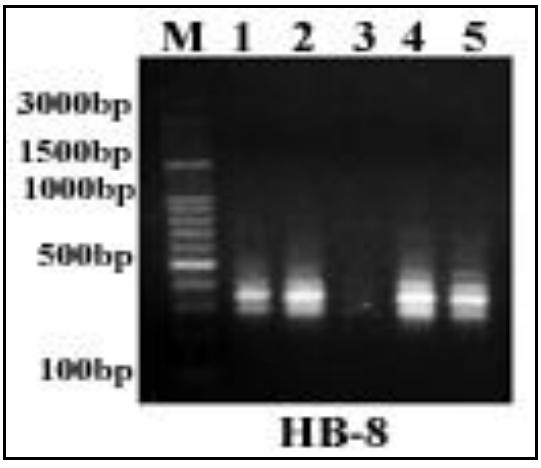

Fig 3: ISSR-PCR produced for gamma irradiated *Earias insulana* adults compared with control using primer HB8.

lane 1=Control, lane 2 =25 Gy, lane 3=50 Gy, lane 4= 100 Gy and lane 5= 200 Gy.

The similarity index indicated a highly drop from 1.00 to 0.50. A major genomic damage could be observed in field population adults resulted from gamma irradiated pupae treated with100 Gy where the similarity index equal 0.50 (Table7). The highest similarity index value was noticed

between control compared with 50 and 200 Gy as well as between 50 and 200Gy where the similarity index recorded 1.00

Table 7: Estimated similarity index between *Earias insulana* adults resulted from pupae irradiated with different doses of gamma radiation using primer HB8:

Treatments	Control	25 Gy	50 Gy	100 Gy	200 Gy
Control	-----	0.80	1.00	0.50	1.00
25 Gy	-----	-----	0.80	0.67	0.80
50 Gy	-----	-----	-----	0.50	1.00
100 Gy	-----	-----	-----	-----	0.50
200 Gy	-----	-----	-----	-----	-----

The ISSR-PCR test of the extracted DNA using primer HB10 revealed high variability between the efficiency associated to genetic variation of the *E. insulana* adults resulted from irradiated pupae. From data presented in Table (8) and depicted in Fig. (5), the formed ISSR-PCR using primer HB10 exposed a total of 5 bands. One common amplified band of 3000 bp was detected in all irradiated adults as well as the control. Only one amplified fragment of 540 bp was detected only in gamma irradiated adult with 50 Gy and disappeared in the other irradiated adults as well as normal ones. Amplified fragment of 840 Gy and 615 Gy was detected only in gamma irradiated adults with 50 Gy and 200 Gy. Amplified band of 470 bp was appeared in control and adults exposed to 50, 100 and 200 Gy of gamma radiation, whereas it was absent in both normal adults and adults irradiated with 25 Gy of gamma radiation. The produced fingerprints from primer HB10 discovered 1 monomorphic, 1 unique and 3 polymorphic bands.

Table 8: Total number and size of ISSR-PCR fragments created by primer HB10 in *Earias insulana* adults resulted from pupae irradiated with different doses of gamma radiation:

MW-bp	MW	Control	25Gy	50Gy	100Gy	200Gy	Polymorphism
Band1	840	…….	……..	840	.....	840	Polymorphic
Band2	615	…….	.....	615	....	615	Polymorphic
Band3	540	…….	.....	540	.-----	……	Unique
Band4	470	……..	……..	470	470	470	polymorphic
Band5	300	300	300	300	300	300	Monomorphic
Total	5	1	1	5	2	4	……

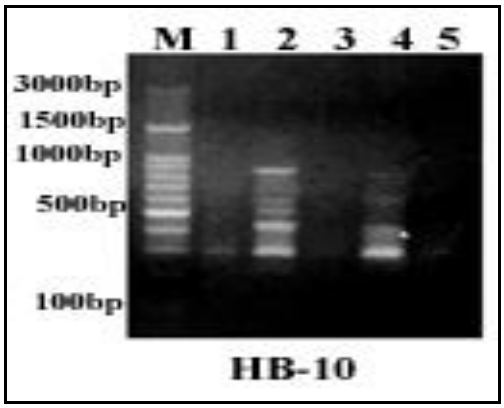

Fig 4: ISSR-PCR produced for gamma irradiated *Earias insulana* adults compared with control using primer HB10.

lane 1=Control, lane 2 =25 Gy, lane 3=50 Gy, lane 4= 100 Gy and lane 5= 200 Gy.

On the light of comparing the analyzed similarity index by using primer HB10, it ranged from 0.33 to 1.00. The highest value was noticed between the normal adults and irradiated adult as full grown pupae with 25 Gy. On the other hand, the lowest value 0.33 between normal adults and adults irradiated as full grown pupae with 50 Gy as well as between adults irradiated with 25 Gy and irradiated adults as pupae with 50 Gy (Table 9).

Table 9: Estimated similarity index between *Earias insulana* adults resulted from pupae irradiated with different doses of gamma radiation using primer HB10.

Treatments	Control	25 Gy	50 Gy	100 Gy	200 Gy
Control	-----	1.00	0.33	0.67	0.40
25 Gy	-----	-----	0.33	0.67	0.0
50 Gy	-----	-----	-----	0.57	0.89
100 Gy	-----	-----	-----	-----	0.67
200 Gy	-----	-----	-----	-----	-----

The ISSR-PCR analysis of the extracted DNA samples using primer HB10 revealed high variability between the efficiency of different gamma irradiated doses under study on genetic variation of the *E. insulana* adults as pupae. The obtained results are presented in Table (8) and illustrated graphically in Fig. (5), the ISSR-PCR pattern produced by amplification of the adult homogenates DNA using primer HB10 contained a total of 7 bands. The number of DNA fragments ranged from 3 to 7 bands. The PCR patterns resulted from using primer HB10 discriminated between the control and the four doses of gamma radiation exposed to the tested pest. Three common amplified bands of 830, 280 and 215 bp were detected in all irradiated adults with different doses of gamma radiation as well as the control. Only one amplified fragment of 620 bp was detected only in both control and gamma irradiated adult with 50 Gy and disappeared in the other irradiated adults. Amplified fragment of 840 Gy and 615 Gy were detected only in gamma irradiated adults with 50 Gy. Amplified band of 450 bp was appeared in control and adults exposed as pupae to 25, 50 and 200 Gy of gamma radiation, whereas it was absent in adults irradiated as pupae with 100 Gy of gamma radiation. The fingerprints generated by primer HB11 revealed three monomorphic and four polymorphic profiles.

Table 10: Total number and size of ISSR-PCR fragments generated by arbitrary primers in *Earias insulana* adults resulted from pupae irradiated with different doses of gamma radiation using primer HB11:

MW-bp	MW	Control	25 Gy	50 GY	100 Gy	200 Gy	Polymorphism
Band1	830	830	830	830	830	830	Monomorphic
Band2	620	620	…….	620	……..	……..	Polymorphic
Band3	530	530	……..	……	…….	530	Polymorphic
Band4	450	450	450	…..	…….	450	Polymorphic
Band5	400	400	400	400.	……..	400	Polymorphic
Band6	280	280	280	280	280	280	Monomorphic
Band7	215	215	215	215	215	215	Monomorphic
Total	7	7	5	5	3	6	……

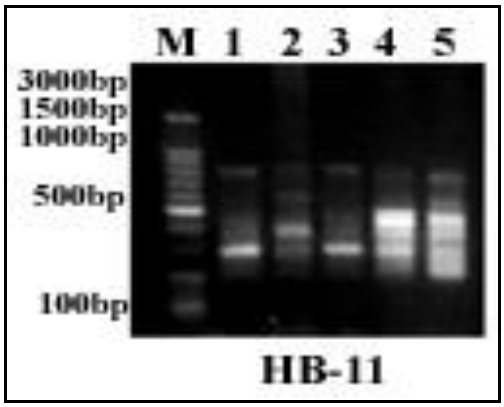

Fig 5: ISSR-PCR produced for gamma irradiated *Earias insulana* adults compared with control using primer HB11.

lane 1=Control, lane 2 =25 Gy, lane 3=50 Gy, lane 4= 100 Gy and lane 5= 200 Gy.

Comparing the analyzed similarity index by using primer HB11, it ranged from 0.67 to 0.92. The highest value 0.92 was noticed between the normal adults and irradiated adults as full grown pupae with 200 Gy. On the other hand, the lowest value 0.67 between adults irradiated as full grown pupae with 100 Gy and adults irradiated as pupae with 200 Gy (Table 11).

Table 11: Estimated similarity index between *Earias insulana* adults resulted from pupae irradiated with different doses of gamma radiation using primer HB11:

Treatments	Control	25 Gy	50 Gy	100 Gy	200 Gy
Control	-----	0.83	0.83	0.60	0.92
25 Gy	-----	-----	0.80	0.75	0.91
50 Gy	-----	-----	-----	0.75	0.72
100 Gy	-----	-----	-----	-----	0.67
200 Gy	-----	-----	-----	-----	-----

The amplified analysis of the extracted DNA samples using primer HB13 demonstrated high variation among the potency of different gamma irradiated doses under study on genetic diversity of the *E. insulana* adults. The obtained results are summarized in Table (12) and depicted graphically in Fig. (6), the ISSR-PCR pattern produced by amplification of the adult homogenates DNA using primer HB10 contained a total of 8 fragments, distributed to 7, 4, 6, 3 and 7 fragments detected in control and gamma irradiated adults as pupae with doses of 25, 50, 100 and 200 Gy; respectively. The molecular weight of the fragments ranged between 145 to 1435 bp. The PCR patterns resulted from using primer HB13 discriminated between the control and the four doses of gamma radiation exposed to the tested pest. Three common amplified bands number 6 and 7 of molecular weight 370 and 260 bp were detected in all irradiated adults with different doses of gamma radiation as well as the control. Only one amplified fragment of 1435 bp was detected only in both gamma irradiated adults as pupae with 50 Gy and 200 Gy; whereas it disappeared in the control and the other irradiated adults. Amplified two fragment of 960 Gy and 145 Gy was detected only in control and gamma irradiated adults with 200 Gy. Amplified band of 845 bp was appeared in control and adults exposed as pupae to 25 and 50 200 Gy of gamma radiation, whereas it was absent in adults irradiated as pupae with 100 Gy and 200 Gy of gamma radiation. The fingerprints generated by primer HB13 revealed two monomorphic and six polymorphic profiles.

Table 12: Total number and size of ISSR-PCR fragments generated by arbitrary primers in *Earias insulana* adults resulted from pupae irradiated with different doses of gamma radiation using primer HB13.

MW-bp	MW	Control	25 Gy	50 Gy	100 Gy	200 Gy	Polymorphism
Band1	1435	……..	……..	1435	……	1435	Polymorphic
Band2	960	960	……..	……	……	960	Polymorphic
Band3	845	845	845	845	.-----	…….	Polymorphic
Band4	700	700	700	700	…….	700	Polymorphic
Band5	460	460	……..	460	460	460	Polymorphic
Band6	370	370	370	370	370	370	Monomorphic
Band7	260	260	260	260	260	260	Monomorphic
Band8	145	145	……	……..	……	145	Polymorphic
Total	8	7	4	6	3	7	……

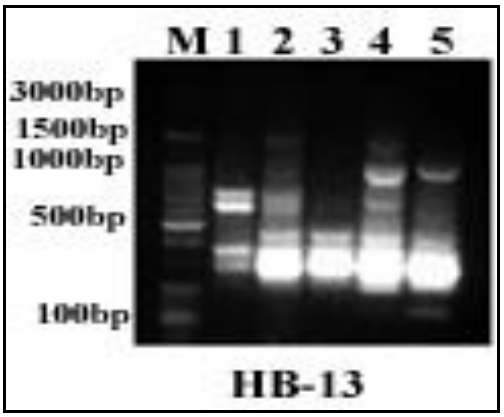

Fig 6: ISSR-PCR produced for gamma irradiated *E. insulana* adults compared with control using primer HB13.

lane 1=Control, lane 2 =25 Gy, lane 3=50 Gy, lane 4= 100 Gy and lane 5= 200 Gy.

Comparing the analyzed similarity index by using primer HB11, it ranged from 0.43 to 0.77. The highest value 0.77 was noticed between the normal adults and irradiated adults as full grown pupae with 50 Gy and between irradiated adults with 50 and 200 Gy. On the other hand, the lowest value 0.43 between and adults irradiated as pupae with 200 Gy (Table 13).

Table 13: Estimated similarity index between *Earias insulana* adults resulted from pupae irradiated with different doses of gamma radiation using primer HB13:

Treatments	Control	25 Gy	50 Gy	100 Gy	200 Gy
Control	-----	0.74	0.77	0.60	0.43
25 Gy	-----	-----	0.80	0.55	0.55
50 Gy	-----	-----	-----	0.67	0.77
100 Gy	-----	-----	-----	-----	0.60
200 Gy	-----	-----	-----	-----	-----

Discussion

After reviewing the results, it can be said that the banding patterns were produced utilising the Random Amplified Polymorphic DNA (RAPD) approach using short (10 bp) oligonucleotide primers of any sequence. These arbitrary sequences were created to screen the entire genome in general, finding any differences between two or more genomes under comparison because they are not specific for a particular gene or DNA sequence. Only when opposing primer sites are approximately bp apart does PCR amplification take place because these primers bind the

homologous region throughout the genome. In a population sample, mutation brought on by any stress (such as exposure to insecticides or gamma radiation) alters the base sequence of primer binding sites, enabling the detection of polymorphism [6]. The evolution of the genome can occur at various rates [11]. So it's feasible that the PCR-amplified areas change more quickly. This procedure enables the simultaneous examination of multiple primers in a single run due to the minimal amount of template utilised in each reaction. It is possible to start PCR cycling overnight, load the products onto a gel, and conduct analysis the next day [12]. The complementarity between a certain primer sequence and an individual's unique template DNA determines the size and number of RAPD markers.

Primers typically have different amplification efficiencies. According to Kantanen *et al*. [13], some primers fail to amplify, while others result in excessively complicated banding patterns. Initial DNA damage depends mostly on radiation exposure, and in cell lines with documented deficiencies in homologous recombination, the initial rate of double-strand break rejoining can seem normal. This suggests that only a portion of DNA repair deficiency phenotypes may be detectable with the comet assay. It is possible to detect changes in DNA repair capability more easily by using low dose rate radiation exposures. Also, a basis for detecting irradiation treatment in a variety of foods and insects could be a radiation-induced change in DNA. The comet assay has shed light on how particular cells react when exposed to ionizing radiation [14]. Although primary injury by ionizing radiation isn't cell-type dependent (hypoxic and thiol-modified cells being important exceptions), maintenance of DNA injury can be affected by cell environment and genotype [3].

In the current study, six arbitrary-sequence primers were used to check the genomic DNA of control and gamma-irradiated adults as pupae for DNA damage or sequence changes. It's possible that variations in the insect's DNA sequence are to blame for a fragment's omission from the RAPD pattern of tested insects. Primary DNA damage depends mostly on radiation exposure, and in cell lines with documented deficiencies in homologous recombination, the initial rate of double-strand break rejoining can seem normal. This suggests that only a portion of DNA repair deficiency phenotypes may be detectable with the comet assay. To determine variations in DNA repair capacity, low dose rate radiation exposures can be used [3].

In the current study, it was discovered that the ISSR-PCR technology requires high-quality DNA as a requirement for reliable results. In gamma-irradiated Earias insula adults as pupae, the largest number of amplified fragments was 8 produced from primer HB13, whereas the lowest number was 3 found from two primers 49B and HB8.

The ISSR-PCR patterns resulted from amplification of DNA of unirradiated as well as gamma irradiated adults of *E. insulana* discovered the deepest rate of similarity index (0.33) which reveals the maximum degree of variation in DNA construction and sequence was documented between the control and gamma irradiated adults as pupae with 50 Gy using primer HB10. The existing results are in harmony with those achieved by Abdel-Baset [15]. She reported that some primers "OPA-13, OPA-15 and OPD-5" are effective to examine variations in the *Pectinophora gossypiella* and *Culex pipiens* DNA that regarded to alterations in them sequences. Moreover, the obtained data are in accordance with those conveyed by Salem [16], who showed that the genomes of pink bollworms not treated and those exposed to a variety of various insecticides used to manage the pest in the field have varied RAPD patterns caused by amplification of DNA structure and sequence.

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