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Genetic variation and sterility percentages in adults of fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae) produced from gamma irradiated pupae

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Abstract

The objective of the present study was to evaluate the molecular variation in adults of the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) irradiated with doses 20, 40, 60 and 80 Gy as full-grown pupae. Results from RAPD-PCR showed that DNA fragments in the irradiated adults as pupae and unirradiated adults under investigation died. In the present study, six primers of uniformed sequences were used to identify the genomic DNA of *S. frugiperda.* The weight of fragments varied from 185 bp to 1480 bp when compared with unirradiated adults. In the study, irradiated adults were contrasted to unirradiated ones to explore any potential variation to the DNA structure that may be induced by gamma radiation implementation. The data revealed polymorphic, monomorphic and unique fragments in the fingerprints generated in the adults resulting from gamma irradiated pupae when compared with the unirradiated adults. Additionally, the highest number of amplified fragments was 28 generated from the two primers, OP-C15 and OP-D1; whereas the lowest number of amplified bands was 16 generated from primer OP-B3 in gamma irradiated adults. Primer OP-C9 showed the lowest value of similarity index 0.28 between unirradiated adults and irradiated adults as pupae with 80 Gy which reflects the highest degree of alteration in DNA sequence and arrangement. Gamma irradiation of the parent generation induced different sterility levels in the offspring, females were more radiosensitive than males. These results are encouraging and indicate that inherited sterility might be used for control of this pest. Large field experiments should be implemented to confirm the laboratory findings.

Introduction

The fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) (FAW) is difficult to control, manage, or eradicate, because it is polyphagous and transboundary, multiplies fast, has a short life cycle and migrates easily, and lacks the diapause growth phase. Fall armyworm, *S. frugiperda* is currently the most damaging crop pest affecting maize (Kumela *et al.*, 2019). It is a polyphagous (Feeds on several hosts) and migratory (Can

spread to other countries) pest that survives on at least 80 plant species (Harrison *et al*., 2019). Typical early FAW infestation signs and symptoms include small "Pin holes" and "Windowpanes", resulting from feeding of the small worms on leaves.

Expansion of molecular markers delivers excellent information on the genetic diversity of the insect and helps to classify pesticide resistant populations to develop appropriate control strategies (Roehrdanz and Degragillier, 2003). Random Amplified Polymorphic DNA Polymerase chain reaction (PCR) has been used to differentiate various animals' species and their resistance strains. Also, this technique is applied to identify different populations within a species. The technique is quick and easy; this detects nucleotide sequence polymorphisms using a single primer of arbitrary nucleotide sequence (Williams *et al.*, 1990). Random Amplified Polymorphic DNA Polymerase chain reaction (PCR) permits the detection of polymorphisms in inter-microsallite loci, using a primer designed from dinucleotide or trinucleotide simple sequence repeats.

Because gamma rays have a shorter wavelength, they have more energy per photon than X-rays. Now, the primary sources of gamma rays used in radiobiological activity are cobalt-60 and cesium-137. At the level of medicinal plant studies, radiation is a good inducer for many useful improvements. Good results were obtained at the level of germination, yield growth parameters and it enhanced active ingredients (Mali *et al*., 2011; Silva *et al*., 2013 and Desal and Rao, 2014).

Therefore, in the current study, molecular variations could be evaluated in the adults resulting from pupae irradiated with four doses of gamma radiation compared with unirradiated adults. Because of heavy reliance on the application of pesticides for pest control throughout the world, many pest species have developed resistance including a number of lepidopterans. One of the promising methods is the use of gamma radiation to affect reproductive capacity and sterility levels (Espo *et al*., 2015; Timbadiya *et al*., 2018 and Zhen *et al*., 2023).

Materials and methods

1. **Rearing and irradiation technique:**

The laboratory strain of fall armyworm, *S. frugiperda* was obtained from Bollworms Research Department, Plant Protection Research Institute. The pest was reared under laboratory conditions without any contamination with insecticides for more than six generations at 27 ±1C and 65- 70% R.H. The standard tested insect used in the bioassay for all experiments was fed daily on fresh castor bean leaves, *Ricinis communis* (L.) in Bollworms Research Department according to the method described by El-Defrawi *et al*. (1964). Full-grown pupae were irradiated with sub sterilizing and sterilizing doses of gamma radiation (20, 40, 60 and 80 Gy) using Gamma Cell-40 (Cesium "Cs^{137}" Irradiation Unit) located at the National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Egypt. The dose rate of the cell was 0.667 rad/sec.

The bulked DNA extraction was performed using DNA Mini prepared Kit (QIAGEN). Isolation protocol of DNA was as follows:

- Adult tissue was grounded with 400 µl of buffer AP1 and 4 ul of Proteinase K were added to the pellet and then vortexed vigorously.
- Mixture was incubated for 10 min at 65ºC and mixed 2-3 times during incubation by inverting tube.
- Then, 130 µl of buffer AP2 was added to the lysate, mixed, and incubated for 5 min on ice.
- Lysate was applied to the QIA shredder spin column sitting in a 2 ml collection tube and centrifuged for 2 min at maximum speed (10.000 rpm).

- Supernatant from step 5 was transferred to a new tube without disturbing the cell-debris pellet. Typically, 450 µl of lysate was recovered.
-Then, 0.5 volume of buffer AP3 and 1 volume of ethanol (96-100%) were added to the cleared lysate and mixed by pipetting.
- Then, 650 µl of the mixture from step 7 was applied through DNeasy Mini spin column setting in a 2 ml collection tube. Then, centrifuged for 1 min at 8000 rpm and flow-through was then discarded.
- DNeasy column was then placed in a new 2 ml collection tube. Then, 500 µl buffer AW was added onto the DNeasy column and centrifuged for 1 min at 8000 rpm.
-Then, 500 µl buffer AW was added to DNeasy column and centrifuged for 2 min at maximum speed (10.000 rpm) to dry the column membrane.
-DNeasy column was then transferred to a 1.5 ml microfuge tube and 100 µl of preheated (65ºC) buffer AE was pipetted directly onto the DNeasy column membrane. Then, incubated for 5 min at room temperature and centrifuged for 1 min at 8000 rpm to elute.
--Elution was repeated once as described. A new microfuge
can be used for first elute. Alternatively, the microfuge tube can be reused for the second elution step to combine the elutes.

2. Polymerase chain reaction (PCR) condition stock solutions:

2.1. 5X Tris-borate (TBE), pH 8.0:

Tris-base	5.40 g
Boric acid	2.75 g
500 mM EDTA, 8.0	0.29 g
H_2O (d.w) up to	100.00ml

2.2. Ethidium bromide:

-The stock solution was prepared by dissolving 1 g of ethidium bromide in 100 ml distilled water and mixed well with magnetic stirrer.
- Transferred to a dark bottle and stored at room temperature.

2.3. Sample loading dye (5x):

Na-EDTA, pH 8.0 (500 mM)	2.00
Glycerol (100%)	5.00
Bromophenol blue (2%)	0.75
H_2O (d.w.)	1.50

PCR was performed in 30-µl volume tubes according to Williams *et al*. (1990) that contained the following:

dNTPs (2.5 mM)	3.00 µl
$MgCl_2$ (25 mM)	3.00 µl
Buffer (10 x)	3.00 µl
Primer (10 pmol)	2.00 µl
Taq DNA polymerse (5U/µl)	0.20 µl
Template DNA (25 ng)	2.00 µl
H_2O (d.w.)	16.80 µl

3. Polymerase chain reaction (PCR) condition Inter Simple Sequence Repeats (ISSR):

The DNA amplifications were performed in an automated thermal cycle (Model Techno 512) programmed for one cycle at 94º C for 4 min followed by 45 cycles of 1 min at 94º C, 1 min at 57º C, and 2 min at 72º C. the reaction was finally stored at 72º C for 10 min.

4. Gel preparation procedure:

4.1. Agarose (1.50 gm) was mixed with (100ml) l x TBE buffer and boiled in microwave.
4.2. Ethidium bromide (5µl) was added to the melted gel after the temperature became 55°C.
4.3. The melted gel was poured in the tray of mini-gel apparatus and comb was inserted immediately, then comb was removed when the gel become hardened.
4.4. The gel was covered by the electrophoretic buffer (1 x TBE).
4.5. DNA amplified product (15 µl) was loaded in each well.
4.6. DNA ladder (100bp) mix was used as standard DNA with molecular weights of 3000, 1500, 1000, 900, 800, 700,600, 500, 400, 300, 200 and 100 bp. The run was performed for about 30 min at 80 V in mini submarine gel BioRad.

5. Data analysis:

Similarity matrices were done using Gel works in ID advanced software UVP-England Program.

Similarity index:

The similarity index was used to compare patterns within as well as between populations. This index reflects the extent of band sharing and calculated as:

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

Where $\mathbf{N_{ab}}$ is number of bands common to individuals a, b.

N_a and N_b are the 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 **(Nei and Li, 1979).**

Table (1): List of the primer names and their nucleotide used in the study for RAPD-PCR procedure.

Primer Name	Sequences
OP-A3	CAG CAC CCA C
OP-A9	CCTTGACGCA
OP-B3	CAT CCC CCT G
OP-C9	CTC ACC GTC C
OP-C15	GAC GGA TCA G
OP-D1	ACC GCG AAG G

Reproductive potential: The pupae collected from the vials were separated by sex. Fall grown pupae were irradiated with doses of 20, 40, 60 and 80 Gy of gamma irradiated and unirradiated pupae. Different crosses were made between irradiated and unirradiated as well as between irradiated adults were made at all doses of gamma radiation. For all crosses, fecundity, fertility, and sterility data were determined. Three replicates were completed. Two pairs of moths/ replicate Crosses were placed inside glass cages (10 cm diameter X 25 cm high) with white paper placed on the inside to serve as an ovipositional substrate. The adults were provided with 10% honey solution through a cotton wick inserted into a small glass vial. A mean number of eggs, hatchability percentages and sterility levels were counted.

Results and discussion

Random Amplified Polymorphic DNA Polymerase chain reaction (PCR) permits detection of polymorphisms in Random Amplified Polymorphic DNA Polymerase chain reaction (RAPD-PCR) permits detection of polymorphisms in the condition of S. *frugiperda* laboratory population adults resulting from gamma irradiated pupae using a primer designed from dinucleotide or trinucleotide simple sequence repeats. Random Amplified Polymorphic DNA Polymerase chain reaction RAPD-PCR fingerprint profiles were generated by using primers on genomic DNA from the condition of *S. frugiperda* laboratory population adults produced from gamma irradiated full grown pupae with 20, 40, 60 and 80 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 S. *frugiperda* laboratory population adults. The total number of produced fragments was 47 distributed as 8, 6, 6, 8, 9 and 10 generated by primers OP-A3, OP-A9, OP-B3, OP-C9, OP-C15 and OP-D1; respectively. The molecular size ranged from 185 bp to 1480 bp. The highest molecular size was detected by using primer OP-B3, and the lowest molecular size appeared in primer OP-A9. In the present investigation, irradiated adults were compared to normal ones to analyze any change in the DNA structure that might be induced by gamma radiation treatments.

The obtained results showed the fingerprints generated in adults resulted from gamma irradiated full grown pupae by each primer, OP-A3, OP-A9, OP-B3, OP-C9, OP-

C15 and OP-D1 revealed polymorphic, monomorphic and unique, profiles for the pest exposed to different doses of gamma radiation, as well as the untreated one. These primers gave good amplification with distinct fragments.

The RAPD analysis of the extracted DNA samples using Primer OP-A3 is illustrated in Tables (2 and 3) and Figure (1) Primer OP-A3 showed 22 bands were detected in the laboratory populations of the pest under the study. The number of produced fragments distributed as 3, 4, 4, 6 and 5 bands in the S. *frugiperda* adults resulted from unirradiated gamma pupae and irradiated gamma pupae with 20, 40, 60 and 80 GY of gamma radiation: respectively. Two fragments of 760, and 730 bp were shared in adults from pupae irradiated with 60 and 80 Gy of gamma radiation. Amplified one fragment of 685 bp was detected in adults emerging from unirradiated pupae as well as adults resulting from pupae irradiated with 40, 60 and 80 Gy of gamma radiation. One fragment of 460 bp was appeared in control adults as well as adults emerge from gamma irradiated pupae with different doses under the study. One fragment of 400 bp was detected only in adults produced from pupae irradiated with 20 and 40 Gy of gamma radiation. One band 300 Pb appeared only in control adults. One fragment of 270 pb was shared in adults which emerged from pupae irradiated with the four doses and absent in control adults. One band of 200 pb appeared only in adults emerging from pupae irradiated with 20 and 60 Gy of gamma radiation. As summarized in Table (2), polymorphism generated by the primer OP-A3 showed 1 monomorphic, 6 polymorphic and 1 unique profile.

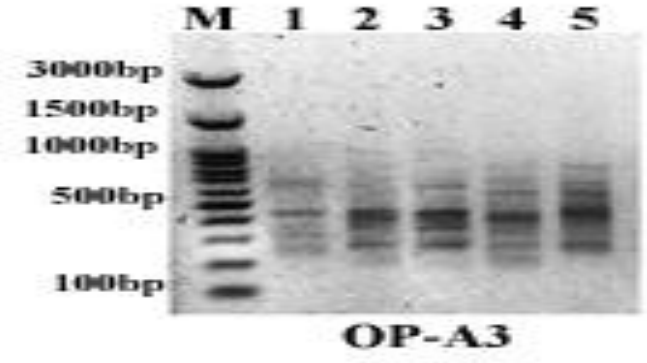

Figure (1): RAPD-PCR produced for adults of S*podoptera frugiperda* resulted from pupae treated with different doses of gamma radiation using primer OP-A3.
Where is lane 1= Lab, lane 2 = 20 Gy, lane 3= 40 Gy, lane 4= 60 Gy and Lane 5= 80 Gy

Table (2): Total number and size of RAPD-PCR fragments generated by arbitrary primers in S*podoptera frugiperda* adults emerged from full grown pupae irradiated with different doses of gamma radiation by using primer OP-A3.

MW-bp	Lab.	20 Gy	40 Gy	60 Gy	80 Gy	Frequency	Polymorphism
Band1	-----	…….	-----	760	760	0.400	Polymorphic
Band2	-----	-----	…….	730	730	0.400	Polymorphic
Band3	685	……	685	685	685	0.800	Polymorphic
Band4	460	460	460	460	460	1.000	Monomorphic
Band5	-----	400	400	-----	-----	0.400	Polymorphic
Band6	300	…..	…..	……	……	0.200	Unique
Band7	-----	270	270	270	270	0.800	Polymorphic
Band8	…..	200	…….	200	……	0.400	Polymorphic
Total	3	4	4	6	5	……	……

As presented in Table (3), the analysis of the similarity index showed low to moderate similarity index values associated with gamma irradiated adults, which ranged from 0.29 to 0.57 compared with the baseline laboratory strain highly indicating the efficacy of gamma irradiation doses in the adults resulted from irradiated full-grown pupae with different doses of gamma radiation. In this respect, high resistance could be noticed in irradiated adults with 20 Gy followed by 60 Gy of gamma radiation, where the estimated similarity index values were 0.29 and 0.44 compared with the laboratory population, respectively.

Table (3): Estimated similarity index between four doses of gamma irradiated adults compared with unirradiated adults by using primer OP-A3.

Doses of gamma radiation	Lab.	20 Gy	40 Gy	60 Gy	80 Gy
Unirradiated	-----	0.29	0.57	0.44	0.50
20 Gy	-----	-----	0.75	0.60	0.44
40 Gy	-----	-----	-----	0.60	0.66
60 Gy	-----	-----	-----	-----	0.54
80 Gy	-----	-----	-----	-----	-----

As shown in Table (4) and Figure (2) the RAPD-PCR pattern produced by amplification of the adult homogenates DNA using primer OP-A9 contained a total of 20 bands. There were 4 bands in the unirradiated adults compared with 6,5, 3 and 2 fragments in irradiated adults with 20, 40, 60 and 80Gy: respectively. Amplified one fragment of 920 bp was shared in control and adults irradiated with 20 Gy. Only one fragment of 715 bp was shared between gamma irradiated adults with 20, 40 and 60 Gy. Two common fragments of 415 and 245 pb were detected in control as well as all irradiated adults with the different doses of gamma radiation. The RAPD- PCR patterns resulted from using primer OP-A9 discriminated one fragment of 340 bp shared between adults irradiated with 20 and 40 Gy doses of gamma radiation exposed to the tested pest. Only one fragment of 185 bp was detected in untreated adults and gamma irradiated adults with 20 and 40 Gy doses. The fingerprints generated by primer OP-A9 revealed four polymorphic and two monomorphic profiles in the tissues of the adults associated with the tested pest.

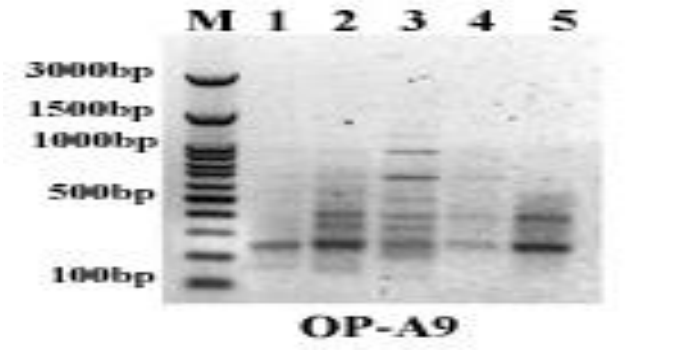

Figure (2): RAPD-PCR produced for adults of S*podoptera frugiperda* resulted from pupae treated with different doses of gamma radiation using primer OP-A9.
Where is lane 1= Lab, lane 2 = 20 Gy, lane 3= 40 Gy, lane 4= 60 Gy and Lane 5= 80 Gy.

Table (4): Total number and size of RAPD-PCR fragments generated by arbitrary primers in *S. frugiperda* adults emerged from full grown pupae irradiated with different doses of gamma radiation by using primer OP-A9.

MW-bp	Unirradiated	20 Gy	40 Gy	60 Gy	80 Gy	Frequency	Polymorphism
Band1	920	920	-----	…….	……	0.400	Polymorphic
Band2	-----	715	715	715	…..	0.600	Polymorphic
Band3	415	415	415	415	415	1.000	Monomorphic
Band4	…...	340	340	……	…..	0.400	Polymorphic
Band5	245	245	245	245	245	1.000	Monomorphic
Band6	185	185	185	……	……	0.600	Polymorphic
Total	4	6	5	3	2	……	……

As illustrated in Table (5), the analysis of the similarity index induced by primer OP-A9 showed low to moderate similarity index values associated with gamma irradiated adults, which ranged from 0.33 to 0.80 compared with the unirradiated adults highly indicating the efficacy of gamma irradiation doses in the adults resulted from irradiated full-grown pupae with different doses of gamma radiation. In this respect, high gene diversity could be noticed in irradiated adults with 80 Gy followed by 60 Gy of gamma radiation, where the estimated similarity index values were 0.33 and 0.57 compared with the unirradiated adults; respectively.

Table (5): Estimated similarity index between four doses of gamma irradiated adults compared with unirradiated adults by using primer OP-A9.

Doses of gamma radiation	Unirradiated	20 Gy	40 Gy	60 Gy	80 Gy
Unirradiated	-----	0.80	0.67	0.57	0.33
20 Gy	-----	-----	0.91	0.67	0.50
40 Gy	-----	-----	-----	0.75	0.57
60 Gy	-----	-----	-----	-----	0.80
80 Gy	-----	-----	-----	-----	-----

As presented in Table (6) and Figure (3) the RAPD-PCR pattern produced by amplification of the adult homogenates DNA using primer OP-B3 contained a total of 16 bands. There were 2 bands in both unirradiated adults and gamma irradiated adults with 20 Gy compared with 5, 4 and 3 fragments in irradiated adults with 40, 60 and 80 Gy; respectively. Amplified one fragment of 1480 bp was detected only in adults irradiated with 40 Gy. Also, only one fragment of 1265 bp appeared in gamma irradiated adults with 80 Gy. One fragment of 815 pb was detected in gamma irradiated adults with 40 and 60 Gy. Common two fragments of 500 and 270 bp were detected in unirradiated adults as well gamma irradiated adults with different doses used in this respect. The fingerprints generated by primer OP-B3 revealed two unique, two polymorphic and two monomorphic profiles in the tissues of the adults associated to the tested pest.

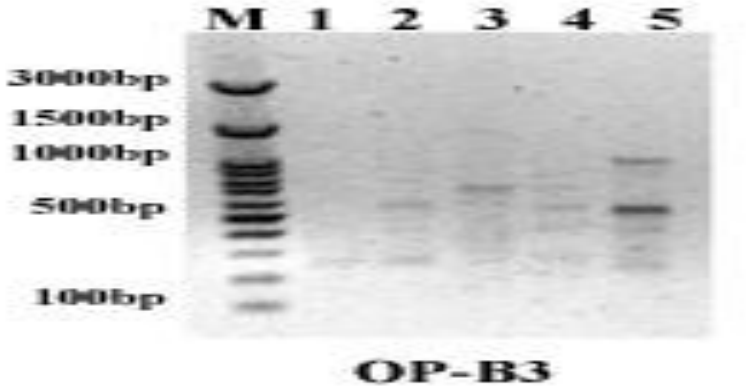

Figure (3): RAPD-PCR produced for adults of S*podoptera frugiperda* resulted from pupae treated with different doses of gamma radiation using primer OP-B3.
Where is lane1=Unirradiated, lane 2= 20Gy, lane 3=40 Gy, lane 4= 60 Gy and Lane 5=80 Gy.

Table (6): Total number and size of RAPD-PCR fragments generated by arbitrary primers in *S. frugiperda* adults emerged from full grown pupae irradiated with different doses of gamma radiation by using primer OP-B3.

MW-bp	Unirradiated	20 Gy	40 Gy	60 Gy	80 Gy	Frequency	Polymorphism
Band1	……	……	1480	…….	……	0.200	Unique
Band2	-----	……	……	……	1265	0.200	Unique
Band3	…..	……	815	815	……	0.400	Polymorphic
Band4	…..	……	630	630	…..	0.400	Polymorphic
Band5	500	500	500	500	500	1.000	Monomorphic
Band6	270	270	270	270	270	1.000	Monomorphic
Total	2	2	5	4	3	……	……

As summarized in Table (7), the analysis of the similarity index showed high and moderate similarity index values associated with gamma irradiated adults, which ranged from 0.57 to 0.80 compared with the unirradiated adults indicating satisfactory the efficacy of gamma irradiation doses in the adults resulting from irradiated full-grown pupae with different doses of gamma radiation by using primer OP-B3. In this respect, highly gene diversity could be noticed in irradiated adults with 40 Gy followed by 60 Gy of gamma radiation, where the estimated similarity index values were 0.57 and 0.67 compared with the unirradiated adults, respectively.

Table (7): Estimated similarity index between four doses of gamma irradiated adults compared with unirradiated adults by using primer OP-B3.

Doses of gamma radiation	Unirradiated	20 Gy	40 Gy	60 Gy	80 Gy
Unirradiated	-----	1.00	0.57	0.67	0.80
20 Gy	-----	-----	0.57	0.67	0.80
40 Gy	-----	-----	-----	0.89	0.50
60 Gy	-----	-----	-----	-----	0.57
80 Gy	-----	-----	-----	-----	-----

Results presented in Table (8) and depicted in Figure (4) revealed that the RAPD-PCR pattern produced by amplification of the adult tissue homogenates DNA using primer OP-C9 contained a total of 19 bands. There were 2 bands in unirradiated adults and 4 fragments in gamma irradiated adults with 20, 40 and 60 Gy compared with 5 fragments in gamma irradiated adults with 80 Gy. Amplified one fragment of 1345 bp was detected only in adults irradiated with 40 Gy. Also, only one fragment of 1050 bp appeared in gamma irradiated adults with 80 Gy. One fragment of 380 pb was detected in unirradiated adults. Also, one fragment of 320 was detected in gamma irradiated adults with 20 Gy. A Common fragment of 280 bp was detected in unirradiated adults as well gamma irradiated adults with different doses used in this respect. The fingerprints generated by primer OP-C9 revealed four unique, three polymorphic and one monomorphic profile in the tissues of the adults associated to the tested pest.

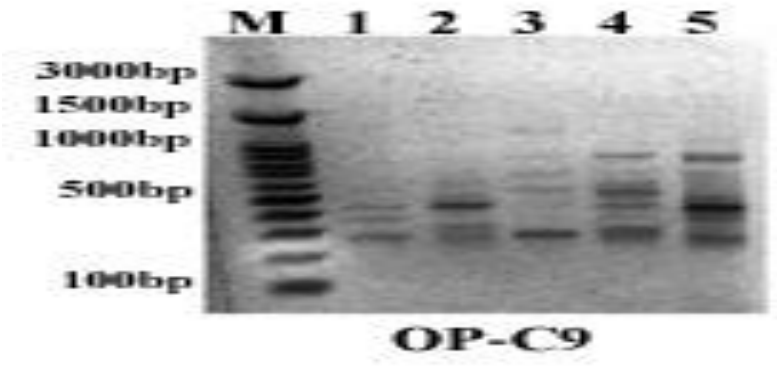

Figure (4): RAPD-PCR produced for adults of S*podoptera frugiperda* resulted from pupae treated with different doses of gamma radiation using primer OP-C9.
Where is lane 1=Unirradiated, lane2= 20Gy, lane3= 40 Gy, lane 4= 60 Gy and Lane 5= 80 Gy.

Table (8): Total number and size of RAPD-PCR fragments generated by arbitrary primers in S*podoptera frugiperda* adults emerged from full grown pupae irradiated with different doses of gamma radiation by using primer OP-C9.

MW-bp	Unirradiated	20 Gy	40 Gy	60 Gy	80 Gy	Frequency	Polymorphism
Band1	-----	…….	1345	…….	…..	0.200	Unique
Band2	-----	-----	…….	……	1050	0.200	Unique
Band3	……	870	870	760	870	0.800	Polymorphic
Band4	……	……	740	740	740	0.600	Polymorphic
Band5	-----	530	…..	-----	530	0.400	Polymorphic
Band6	380	……	…..	……	……	0.200	Unique
Band7	-----	320	…..	……..	……	0.200	Unique
Band8	280	280	280	280	280	1.000	Monomorphic
Total	2	4	4	4	5	……	……

In this investigation, high gene variation could be noticed in gamma irradiated adults with 80 Gy followed by 20, 40 and 60 Gy of gamma radiation, where the estimated similarity index values were 0.28, 0.33, 0.33 and 0.33as compared with the unirradiated adults; respectively. The estimated similarity index values between gamma irradiated adults ranged from 0.33 to 0.75 indicating that gamma irradiation induced damage in genomic structure and sequences (Table 9).

Table (9): Estimated similarity index between four doses of gamma irradiated adults compared with unirradiated adults by using primer OP-C9.

Doses of gamma radiation	Unirradiated	20 Gy	40 Gy	60 Gy	80 Gy
Unirradiated	-----	0.33	0.33	0.33	0.28
20 Gy	-----	-----	0.50	0.75	0.67
40 Gy	-----	-----	-----	0.75	0.67
60 Gy	-----	-----	-----	-----	0.44
80 Gy	-----	-----	-----	-----	-----

As illustrated in Table (10) and Figure (5) demonstrated that the RAPD-PCR pattern generated by amplification of the adult homogenates DNA using primer OP-C15 produced a total of 28 bands. There were 5 bands in both unirradiated adults and gamma irradiated adults with 20 Gy, 7 bands in gamma irradiated adults with 40 and 60 Gy compared and 4 fragments in gamma irradiated adults with 80 Gy. Amplified one fragment of 1270 bp was detected only in unirradiated adults. Also, only one fragment of 1185 bp appeared in gamma irradiated adults with 40 Gy. One fragment of 1060 pb was detected in unirradiated adults as well as gamma irradiated adults with 20, 40 and 60 Gy. Two bands of 840 and 700 bp were detected in both gammas irradiated adults with 40 and 60 Gy. One band of 600 bp appeared in unirradiated adults as well as gamma irradiated adults with 20, 60 and 80 Gy. Common one or two fragments of 570 and 380 bp were detected in unirradiated adults as well gamma irradiated adults with different doses used in this respect. One band of 270 bp was detected in gamma irradiated adults with 20, 40, 60 and 80 Gy. The fingerprints generated by primer OP-C15 revealed two unique, five polymorphic and two monomorphic profiles in the tissues of the adults associated with the tested pest.

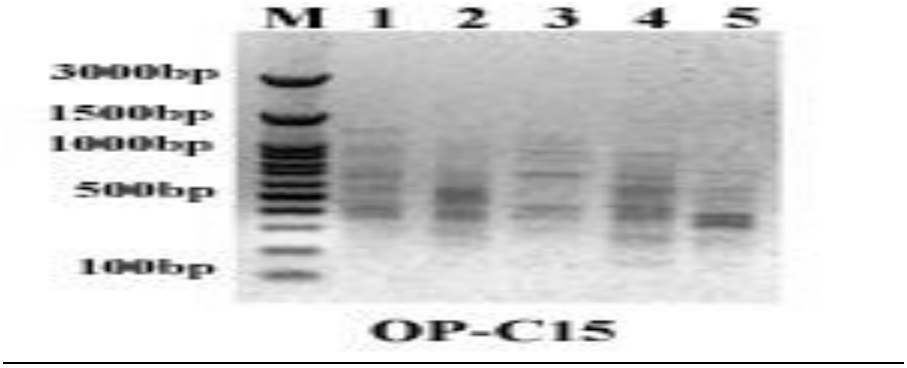

Figure (5): RAPD-PCR produced for adults of S*podoptera frugiperda* resulted from pupae treated with different doses of gamma radiation using primer OP-C15.
Where is lane 1=Unirradiated, lane 2= 20 Gy, lane 3= 40 Gy, lane 4=60 Gy and Lane 5= 80 G

Results presented in Table (11) showed that moderate gene variation could be noticed in gamma irradiated adults with 40 Gy followed by 60 and 80 Gy of gamma radiation, where the estimated similarity index values were 0.50, 0.67, and 0.67 as compared with the unirradiated adults; respectively. The estimated similarity index values between gamma irradiated adults ranged from 0.54 to 0.89 indicating that gamma irradiation induced moderate damage in genomic structure and sequences.

Table (10): Total number and size of RAPD-PCR fragments generated by arbitrary primers in S*podoptera frugiperda* adults emerged from full grown pupae irradiated with different doses of gamma radiation by using primer OP-C15.

MW-bp	Unirradiated	20 Gy	40 Gy	60 Gy	80 Gy	Frequency	Polymorphism
Band1	1270	…….	……	-----	-----	0.200	Unique
Band2	-----	-----	1185	-----	-----	0.200	Unique
Band3	1060	1060	1060	1060	-----	0.800	Polymorphic
Band4	……	-----	840	840	-----	0.400	Polymorphic
Band5	-----	-----	700	700	-----	0.400	Polymorphic
Band6	600	600	……	600	600	0.800	Polymorphic
Band7	570	570	570	570	570	1.000	Monomorphic
Band8	380	380	380	380	380	1.000	Monomorphic
Band9	…..	270	270	270	270	0.800	Polymorphic
Total	5	5	7	7	4	……..	……

Table (11): Estimated similarity index between four doses of gamma irradiated adults compared with unirradiated adults by using primer OP-C15.

Doses of gamma radiation	Unirradiated	20 Gy	40 Gy	60 Gy	80 Gy
Unirradiated	-----	0.80	0.50	0.67	0.67
20 Gy	-----	-----	0.67	0.83	0.89
40 Gy	-----	-----	-----	0.86	0.54
60 Gy	-----	-----	-----	-----	0.73
80 Gy	-----	-----	-----	-----	-----

Data presented in Table (12) and Figure (6) cleared that the RAPD-PCR pattern generated by amplification of the adult homogenates DNA using primer OP-D1 produced a total of 28 bands. There were 7 fragments in both unirradiated adults and gamma irradiated adults with 60 Gy. There were 6, 4 and 4 bands in gamma irradiated adults with 20, 40 and 80 Gy; respectively. Amplified two common fragments of 1340 and 670 bp were detected in unirradiated adults as well as the gamma irradiated adults with 20, 40, 60 and 80 Gy. Two fragments of 1045 and 540 bp appeared in unirradiated adults as well as gamma irradiated adults with 20 and 60 Gy. One band of 910 appeared only in gamma irradiated adults with 80 Gy. Also, one fragment of 825 bp was noticed only in gamma irradiated adults with 60 Gy. One band of 760 bp was observed only in unirradiated adults. Also, one band of 245 bp was observed only in gamma irradiated adults with 40 Gy. One fragment of 480 bp was detected in unirradiated adults and gamma irradiated adults with 20, 40 and 60 Gy. Also, one fragment of 300 bp appeared in unirradiated adults as well as gamma irradiated adults with 20, 60 and 80 Gy. The fingerprints generated by primer OP-D1 revealed four unique, four polymorphic and two monomorphic profiles in the tissues of the adults associated with the tested pest.

Results presented in Table (13) showed that moderate gene diversity could be noticed in gamma irradiated adults with 60 Gy followed by 40 and 80 Gy of gamma radiation, where the estimated similarity index values were 0.43, 0.54, and 0.54 as compared with the unirradiated adults; respectively. The estimated similarity index values between gamma irradiated adults ranged from 0.54 to 0.92 indicating that gamma irradiation induced moderate damage in genomic DNA structure and sequences.

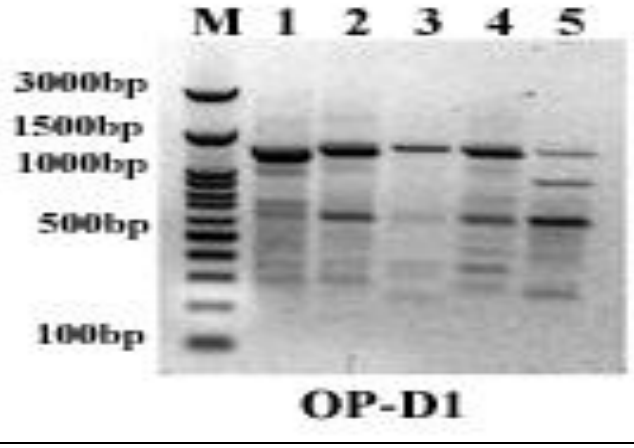

Figure (6): RAPD-PCR produced for adults of S*podoptera frugiperda* resulted from pupae treated with different doses of gamma radiation using primer OP-D1.
Where is lane 1=Unirradiated, lane 2= 20 Gy, lane 3= 40 Gy, lane 4=60 Gy and Lane 5= 80 G

Table (12): Total number and size of RAPD-PCR fragments generated by arbitrary primers in S*podoptera frugiperda* adults emerged from full grown pupae irradiated with different doses of gamma radiation by using primer OP-D1.

MW-bp	unirradiated	20 Gy	40 Gy	60 Gy	80 Gy	Frequency	Polymorphism
Band1	1340	1340	1340	1340	1340	1.000	Monomorphic
Band2	1045	1045	…….	1045	-----	0.600	Polymorphic
Band3	…..	-----	-----	-----	910	0.200	Unique
Band4	-----	-----	-----	815	……	0.200	Unique
Band5	760	……	-----	-----	-----	0.200	Unique
Band6	670	670	670	670	670	1.000	Monomorphic
Band7	540	540	-----	540	……	0.600	Polymorphic
Band8	480	480	480	480	-----	0.800	Polymorphic
Band9	300	300	-----	300	300	0.800	Polymorphic
Band10	-----	…..	245	-----	-----	0.200	Unique
Total	7	6	4	7	4	0.200	--------

Table (13): Estimated similarity index between four doses of gamma irradiated adults compared with unirradiated adults by using primer OP-D1.

Doses of gamma radiation	Unirradiated	20 Gy	40 Gy	60 Gy	80 Gy
Unirradiated	-----	0.92	0.54	0.43	0.54
20 Gy	-----	-----	0.60	0.92	0.60
40 Gy	-----	-----	-----	0.54	0.60
60 Gy	-----	-----	-----	-----	0.54
80 Gy	-----	-----	-----	-----	-----

Reviewing the aforementioned results, it could be concluded that the banding patterns are created using short oligonucleotide primers (10bp in length) of arbitrary sequence in Random Amplified polymorphic DNA (RAPD) technique. These arbitrary sequences are not specific for a particular gene or DNA sequence, so they are designed to screen the whole genome in general detecting any changes between two or more genomes under comparison. These primers bind the homologous sequence along the genome and PCR amplification only occurs when opposing primer sites are about bp apart. Within a population sample, mutation caused by any stress (e.g.

Insecticide treatment or gamma radiation) changes the base sequence of primer binding sites, allowing polymorphism to be detected (Williams *et al.*, 1990). Different parts of the genome can evolve at different rates (Nei, 1987).

Therefore, it is possible that the regions amplified by PCR evolve at a higher rate. Because of the small number of templates used in each reaction, this protocol allows for the simultaneous analysis of several primers in a single run. PCR cycling may be initiated overnight, and the products are loaded into a gel and analyzed the following day (Yoder *et al*., 1999). The number and size of RAPD markers depend on the complementary sequence of a particular primer and template DNA which is characteristic of an individual.

Primers usually do not have the same amplification efficiency. Kantanen *et al*. (1995) found that some primers fail to amplify; others produce too complex banding patterns. Initial DNA damage is dependent primarily on radiation dose and the initial rate of rejoining of double-strand breaks can appear normal for cell lines that are known to be deficient in homologous recombination. This means that the comet assay may be able to identify only a subset of DNA repair deficiency phenotypes. Using low dose rate radiation exposures can enhance the ability to detect differences in DNA repair capacity. Imamura *et al*. (2004) mentioned that radiation- induced change in DNA could be a basis for detection of irradiation treatment in a number of foods and insects. The comet assay has provided useful insights into the behavior of individual cells exposed to ionizing radiation. Although initial damage by ionizing radiation is largely cell-type independent (hypoxic and thiol-modified cells being important exceptions), repair of DNA damage can be influenced by genotype and cell environment (Sayed and El-Ghobary, 2019).

In the present investigation, the genomic DNA of the control and gamma irradiated adults as pupae were screened for DNA damage or sequence changes using six primers of arbitrary sequences. The absence of a fragment from the RAPD pattern of tested insects may be due to changes in DNA sequence of the insect under investigation. Initial DNA damage is dependent primarily on radiation dose and the initial rate of rejoining of double-strand breaks can appear normal for cell lines that are known to be deficient in homologous recombination. This means that the comet assay may be able to identify only a subset of DNA repair deficiency phenotypes. Using low dose rate radiation exposures can enhance the ability to detect differences in DNA repair capacity (Sayed and El-Ghobary, 2019).

In the present work it was found that DNA of good quality is a prerequisite to having reproducible results from RAPD-PCR technique. The highest number of amplified fragments was 30 generated from primer OP-A9, whereas the lowest number of amplified fragments were 6 detected from two primer OP-B3 in gamma irradiated *S. frugiperda* adults as pupae.

The RAPD-PCR patterns resulted from the amplification of DNA of unirradiated as well as gamma irradiated adults of *S. frugiperda* revealed the lowest value of similarity index (0.28, 0.33, 0.33 and) which reflects the highest degree of change in DNA structure and sequence was recorded between the genomes of the control and gamma irradiated adults as pupae with 80, 20, 40 and 60 Gy; respectively using primer OP-C9. The obtained results revealed that primers OP-A9, OP-C15, Op-C9 and OP-DI are strong tools to investigate changes in the *S. frugiperda* genomic DNA, probably due to changes in sequences of these primers. The sequence of the primer is known to be the factor for screening DNA or polymorphism. These results are in accordance with those

obtained by Abdel-Baset (2009), the author revealed that primers OPA-13, OPA-15 and OPD-5 are important to study changes in the *P. gossypiella* and *Culex pipiens* genomic DNA, probably due to changes in sequences of these primers. The sequence of the primer is known to be the factor for screening DNA or polymorphism. The results agree with those reported by Salem (2018), who demonstrated that the RAPD patterns resulted from the amplification of DNA structure and sequence were different between the genomes of untreated pink bollworm and those exposed to a wide spread of different insecticides used to control the pest in the field.

Based on reproductive potential, Tapozada *et al.* (1966) proposed the following equation for calculating the sterility percentages as follow:

Sterility % =100 – (axb /AxB) x 100

Where:

a = Mean number of eggs laid / female in the treatment

b = % hatch in the treatment

A = Mean number of eggs laid / female in the control

B = % hatch in the control

For reproductive potential and sterility, results illustrated in Table (14) indicated that the effect of radiation on the number of eggs laid by irradiated females mated with unirradiated males was greatest at doses above 20 Gy. Females irradiated with higher doses showed a marker decrease in the number of eggs when compared with the control. At a dose of 80 Gy, fewer eggs were laid by the irradiated female. Fertility percentages in eggs laid by irradiated females with doses between 20 and 60 Gy decreased gradually and reached at 80 Gy. The obtained results are in accordance with those reported by other authors working on fall armyworm (Espo *et al.* 2015; Timbadiya *et al*., 2018 and Zhen *et al*., 2023).

Table (14): Effect of selected doses of gamma radiation applied to fall grown pupae of *Spodoptera frugiperda* on sterility percentages of the resulted adult.

Doses of gamma radiation (Gy)	Crosses	Mean NO.of total eggs/female	Hatchability %	Sterility %
0	♂ U x ♀ U	1830	91.9	0
20	♂ I x ♀ U	1540	68.8	36.59
	♂ U x ♀ I	1468	54.6	52.03
	♂ I x ♀ I	1069	32.2	79.40
40	♂ I x ♀ U	1220	58.5	57.28
	♂ U x ♀ I	992	40.4	76.01
	♂ I x ♀ I	768	28.8	86.76
60	♂ I x ♀ U	1090	29.5	80.75
	♂ U x ♀ I	888	18.4	90.22
	♂ I x ♀ I	312	0.0	100
80	♂ I x ♀ U	301	0.0	100
	♂ U x ♀ I	92	0.0	100
	♂ I x ♀ I	0.0	0.0	100

Where is. U = unirradiated moth I = irradiated moth

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