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Evaluation of Changes in Genetic Characteristics of Male Peach Fruit Fly, *Bactrocera zonata* Irradiated with Gamma Radiation using RAPD-PCR Technique

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ABSTRACT

Full grown male pupae of the peach or guava fruit fly, *Bactrocera zonata* were irradiated with sterilizing and substerilizing doses of gamma radiation. The effect of gamma rays on the DNA patterns of adult males showed alterations from the control. Exposure to radiation caused very frequently the appearance of some extra bands and the deficiency of others in the RAPD-PCR amplification patterns of the irradiated insects.

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All authors designed the study and performed the experimental work. RMS analyzed the data. NFZ and AFH wrote and revised the manuscript.

Key words
Bactrocera zonata, Gamma radiation, Random amplified polymorphic DNA, Fingerprints RAPD.

INTRODUCTION

Peach fruit fly, *Bactrocera zonata* Saunders (Diptera: Tephritidae) caused a severe damage to a wide range of fruits. The main hosts of *B. zonata* are guava, mango and peach. Secondary hosts include apricot, fig and citrus. This insect also has been recorded on over 50 cultivated and wild plant species, mainly those with fleshy fruits (OEPP/ EPPO, 2005).

From decades earlier, producers and consumers have been trying to prevent and eliminate infestation of food commodities by using chemical insecticides. However, the extensive use of these insecticides has directly led to develop insect resistance to them and presence of undesirable residues which are considered a potential health hazard. Therefore, irradiation technique seems to offer a suitable solution, for its effectiveness and safety (Subramanyam and Hagstrum, 1995).

Investigations into the possibility of using radiation in sterilizing insect males (SIM) were initiated by Muller (1950) on *Drosophila.* Many programs were developed on using SIM in pest control and the most successful one was the eradication of the screw worm fly, *Cochliomyia hominivorax* from the island of Curacao, U.S.A. (Baumhover *et al*., 1955; Lindquist, 1955; Knipling, 1960). Currently, the SIM is the most widely applied against tephritid fruit flies (Enkerlin, 2005).

The prosperity of SIM as pest control method has resulted from its species-specificity and can be used in integration with other control methods. In addition, released gamma- sterilized males are mobile and would actively compete with normal males (Hendrichs *et al*., 2007).

The random amplified polymorphic DNA (RAPD) assay based on PCR, developed by Williams *et al*. (1990), amplifies random DNA fragments with short primers of arbitrary nucleotide sequence. This technique has been reported as an effective method for species classification, genetic mapping and phylogeny studies. Since the random amplified polymorphic DNA has been used as a powerful tool for detecting DNA damage and mutations (Savva, 1998; Atienzar *et al*., 2001; Ercan, 2015). Therefore, any change can be related to the induced sterility and can be used as bio-indicator for sterility in the mass rearing of *B. Zonata* for sterile male release.

The intent of this study was to find a genetic marker made by comparing the DNA patterns of normal and irradiated adult male which would serve as sterility fingerprint and to study the inheritance of sterility.

MATERIALS AND METHODS

Rearing of insect and irradiation

The original colony of Peach fruit fly, *Bactrocera zonata* was obtained from Natural Products Department, National Center for Radiation Research and Technology

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(NCRRT), Cairo, Egypt. The insect was reared under laboratory conditions of 25± 2ºC temperature; and 60± 5% relative humidity. Full grown pupae were irradiated with substerilizing and sterilizing doses of gamma radiation (70 and 90Gy) which has been previously reported by Zahran *et al*. (2013). The irradiation technique was performed using Gamma Cell-40 (Cesium-137 Irradiation Unit) at NCRRT. The dose rate was 0.714 rad/sec at the time of experimental research.

RAPD-PCR amplification

Tissue samples were ground with liquid nitrogen using a mortar and pestle. Extraction and purification of samples were carried out with use of DNeasy mini spin columns as described by Qiagene handbook manufacturer manual and stored at -80ºC. RAPD-PCR reaction was performed according to the protocol of Williams *et al*. (1990). Reactions were performed in a total volume 50µL reaction buffer (100mMKcL, Tris HCL ph 8.3) 3mM MgCl2, 200 mMdNTPs (Promega Biotech. Inc.) 50 p/mole primers and 1µTaq polymerase. This reaction was added to 0.1µl genomic DNA .Tubes containing mixes were placed in a thermocycler (Perkin–Elmer 2400) and DNA was amplified using the following temperature profile (modified from Black *et al*., 1992). The 4 random primers used in the study were obtained from Operon Technologies with the sequences tabulated in Table I.

Table I.- The nucleotide sequences of the primers used for RAPD-PCR analysis.

Primer number	Primer name	Sequence
P1	28SS	5'- GAC CCG TCT TGA AMC AMG G -3'
P2	28SA	5'- TCG GAR GGA ACC AGC TAC TA -3'
P3	SxL1-F	5'- CAT ACG GAT ACA ATG GTT AT -3'
P4	Ace	5'- CGG CAA GTT GAA CGA GAG -3'

Amplification was carried out for 30 cycles. After the reaction, mixture was mixed with DNA loading buffer and electrophoresed on 1% Agarose gel. The temperature profiles were 94°C for 5 min, 94°C for 40 sec, 72°C for 2 min, 36°C for 1 min, 72°C for 7 min and 4°C for hold.

Data analysis

The gel image was recorded using a gel Documentation system (UVP,UK). Bands in each treatment were analyzed using the similarity index formula of Nei and Li (1979). The index reflects the extent of band sharing between individuals. CLIQS 1D PRO (Core Laboratory Image Quantification Software professional) supported by Total lab (Newcastle, England).

RESULTS

RAPD Finger print profiles were generated by using 10- per primers on genomic DNA from the adults *B. zonata*. Four random primers 28SS, 28SA, SxL1-F and Ace were used for screening the genomic DNA extracted from unirradiated males (control) and males irradiated with 70 and 90Gy. Banding patterns for that used four random primers were scored as present (x) or absent (-) according to their molecular sizes.

Table II revealed that the percentage of polymorphism were 83.33, 71.42, 75 and 66.67 when using 28SS, 28SA, SxL1-F and Ace primers, respectively. The number of produced fragments was 40 distributed as 12, 7, 12 and 9 with 28SS, 28SA, SxL1-F and Ace, respectively. The molecular size of the fragments ranged from 1414.41bp to 144.79bp. The highest and the lowest molecular size were detected by using primer 28SS. In the present investigation irradiated males were compared to normal ones by analyzing the DNA structural changes which might be induced by irradiation treatment.

Table II.- A list of the numbers of polymorphic and monomorphic bands of the different treatments with the listed four random primers.

Primer	Polymorphic bands	Monomorphic bands	Total	%polymorphism
28SS	10	2	12	83.33
28SA	5	2	7	71.42
SxL1-F	9	3	12	75
Ace	6	3	9	66.67
Total	30	10	40	-

Table III and Figure 1 show that RAPD markers using primer 1 (28SS) indicate the presence of only 2 common bands in normal and irradiated *B. zonata*. In addition, appearance of 2 new bands and disappearance of 7 bands in adults of irradiated pupae in comparison to unirradiated control.

RAPD analysis using primer 2 (28SA) indicated the presence of 5 bands in control; 2 bands of them were present in irradiated samples and the other 3 bands disappeared in irradiated samples (Table III). Figure 1 revealed occurrence of 1 new band in adults of pupae irradiated with 90 Gy and another one in the two irradiated samples when using primer 2 (28SA).

The results of RAPD analysis using primer 3 (SxL1-F) announced disappearance of 7 bands from irradiated samples, and appearance of 2 new bands in adults resulted from irradiated pupae with 70Gy in comparison to control

(Table III, Fig. 1). After irradiation with 90Gy; 3 bands were common with control and 70Gy.

By using of primer 4 (Ace) six bands were observed in control, 3 of them disappeared in males irradiated as pupae (a gene was turned off) and the other 3 bands were common with the irradiated males. The fingerprint implied appearance of 3 new bands; 2 bands in males irradiated as pupae with 70 Gy and 1 band in both irradiated samples with 70 and 90Gy (Table III, Fig. 1).

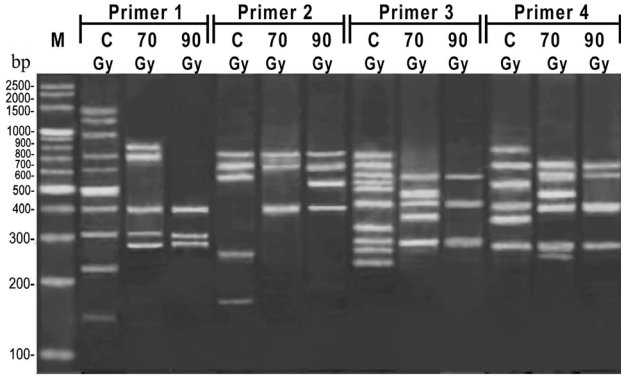

Fig. 1. RAPD-PCR pattern resulting from amplification of genomic DNA of *B. zonata* male.

Table IV and Figure 2 illustrated that the highest similarity values were found between the adult males irradiated as pupae with 70 and 90Gy for the 4 tested primers. On the other hand, the lowest values were between the control and the adult males irradiated as pupae with 90Gy for primers 28SS and 28SA, and between the control and the adult males irradiated as pupae with 70Gy for primers SxL1-F and Ace.

On comparing the analyzed similarity matrices by using the 4 primer; the highest value (0.86) was found between the adults' male irradiated as pupae with 70Gy and 90Gy when using primer 2. In contrast, the lowest value (0.31) was between control and the adults' male irradiated as pupae with 90Gy when using primer 1.

DISCUSSION

The chemical damage to organic molecules from the absorbing medium through which the radiation pass can be caused directly (mostly by particulate types of radiation) or indirectly by free radicals (*i.e.,* atoms or molecules carrying at least one unpaired orbital electron in the outer shell), secondary electrons, or other charged particles (Hall and Giaccia, 2006). The radio induced ions and radicals, most of them coming from the water radiolysis, may react with neighboring molecules to produce

Table III.- DNA polymorphism using randomly amplifying DNA (RAPD) with four primers for *Bactrocera zonata* male (unirradiated and irradiated as full grown pupae with 70Gy and 90Gy).

Band No.	Molecular size (bp)	Control	70Gy	90 Gy
Primer 1 (28SS)				
1	1414.41	x	-	-
2	1214.34	x	-	-
3	956.68	x	-	-
4	800	-	x	-
5	721.29	x	x	-
6	611.68	x	-	-
7	485.31	x	-	-
8	394.98	x	x	x
9	305.81	x	x	x
10	284.26	-	x	x
11	223.61	x	-	-
12	144.79	x	-	-
Primer 2 (28SA)				
1	736.75	x	x	x
2	637.85	x	x	x
3	565.27	x	-	-
4	526.01	-	-	x
5	398.48	-	x	x
6	257.54	x	-	-
7	167.4	x	-	-
Primer 3 (SxL1-F)				
1	730.62	x	-	-
2	644.1	x	-	-
3	566.96	x	x	x
4	535.52	x	-	-
5	500	x	-	-
6	468.97	-	x	-
7	423.8	x	x	x
8	368.88	-	x	-
9	329.48	x	-	-
10	285.65	x	x	x
11	268.36	x	-	-
12	238.79	x	-	-
Primer 4 (Ace)				
1	781.2	x	-	-
2	641.5	x	x	x
3	576.7	-	x	x
4	522.25	x	-	-
5	474.39	-	x	-
6	408.29	x	x	x
7	361.13	x	-	-
8	277.14	x	x	x
9	252.71	-	x	-

Table IV.- Similarity index among *Bactrocera zonata* male DNA pattern using UPGAMA (Dice) method.

Lane	Control	70Gy	90Gy
Primer 1 (28SS)			
Control	1	0.40	0.31
70Gy		1	0.75
90Gy			1
Primer 2 (28SA)			
Control	1	0.5	0.44
70Gy		1	0.86
90Gy			1
Primer 3 (SxL1-F)			
Control	1	0.4	0.46
70Gy		1	0.75
90Gy			1
Primer 4 (Ace)			
Control	1	0.5	0.6
70Gy		1	0.8
90Gy			1

secondary DNA radicals or even chain reactions, particularly in lipids. Most significant biological effects result from damage to DNA, which is the critical target in living organisms. Some radio induced lesions in DNA are single-strand breaks in the phosphodiester linkage, double-strand breaks, base damage, protein–DNA cross-links, and protein–protein cross-links.

The double-strand breaks in DNA double helix are believed to be the most important type of lesion produced in chromosome by ionizing radiation, cracking the chromatin into different pieces that may result in cell killing or mutation. Examples of lethal aberrations to the cell are the dicentric and ring (which are chromosome aberrations) and the anaphase bridge (a chromatid aberration). Two relevant aberrations that are usually not lethal to the cell are symmetrical translocation and small deletions. These changes and mutations left in the genetic code will influence base pairing, coding, transcription, and gene expression (O'Brien and Wolfe, 1964; Hall and Giaccia, 2006).

The study of the RAPD–PCR profiles of normal and irradiated males as pupae of *Bactrocera zonata s*howed variation between them. The appearance of some extra bands and the disappearance of others, as a result of irradiation, were recorded throughout this study causing variations in the PCR patterns among different samples.

These induced variations differ according to the dose of irradiation given and differ from unirradiated males and males irradiated as pupae. These results agree with those of Lea (1956) who stated that ionizing radiations deposits energy in an absorber at discrete loci randomly

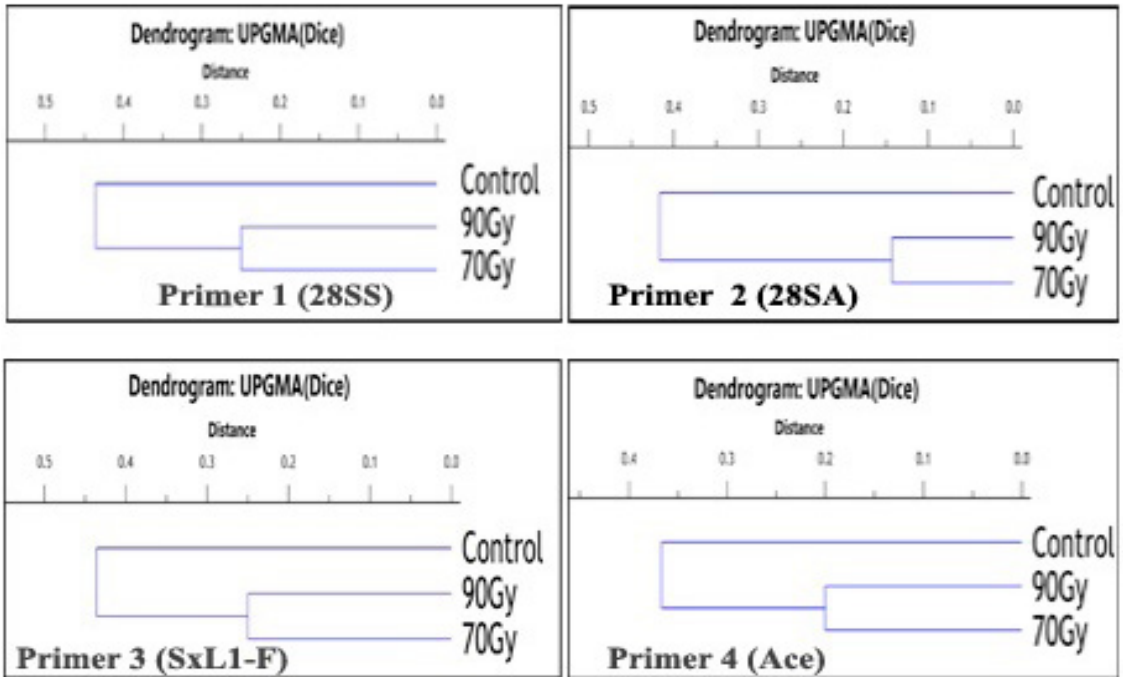

Fig. 2. Phylogenetic tree using UPGAMA method.

distributed within the radiation field and that ionization which occurs in the cellular structure depends on the dose of radiation and on target size. In this study the RAPD–PCR profile of some irradiated samples, revealed the appearance of some extra fragments and the disappearance of one or more fragments. Similar findings were recorded by Alexandrov *et al*. (1998) who stated that 62.5% of the gamma rays induced mutants revealed deficiency of one or more fragments produced , the rest of mutants showed no alterations in the PCR patterns indicating possible small scale changes (point mutations) inside the gene region studies or the gross lesion probably situated. Similar results on *Callosobruchus maculatus* were also obtained by Zaghloul *et al*. (2006) and Hamed *et al*. (2009).

Alterations in DNA patterns of the sterile males provide an explanation for the induced sterility and augment the importance of the DNA in the transfer of genetic materials from cell to cell. However, the similarity in DNA patterns of normal and irradiated adult was interpreted by supposing that radiation induced damages in regions of the genome other than at the loci under study. Also, El-Said (2013) on her study on *Spodoptera littoralis* males (emerged from irradiated pupae) at various generations (parents, F_1 and F_2) showed variation between them. The appearance of extra bands and disappearance of others, as a result of irradiation, were recorded throughout the investigation causing the variations in the PCR pattern among the different samples although they are belonging to the same generation. The variations in the PCR patterns depend on the primer used, the dose of gamma irradiation given to the insect, the kind of the irradiation source and differ from parent, F_1 and F_2.

Exposure of *B. zonata* male pupae to gamma rays may result in the formation of covalently bound adducts between the chemical or its metabolites and the DNA faulty repair of these adducts often results in mutations and, sometimes, cytogenetic changes which lead to degrees of sterility depending on the radiation dose.

CONCLUSION

From aforementioned results, it could be concluded that RAPD assay is a potent tool to distinguish between the sterilized and non sterilized adult fly.

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Conflict of interest statement

We declare that we have no conflict of interest.

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