Evaluation of cytogenotoxic effects of X-rays on *Vicia faba* plant

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Abstract: The cytogenotoxic effects of different X-rays doses (54, 108, 162 and 216 Kelo Volt) on *Viciafaba* plant were evaluated by measuring cytological analysis (in mitotic division) and estimating changes in DNA by using RAPD-PCR analysis. The results showed that the two X-rays treatments (54 and 216 K.V.) caused a marked reduction in mitotic index. On the other hand, all X-rays treatments induced unbalance of mitotic stages percentage. Abnormalities were observed at different treatments and the percentage of this value increased by increasing of X-rays doseexcept for the 108 K.V. X-rays treatment. The most dominant abnormalities were: stickiness, C-metaphase, disturbed and bridges. Other abnormalities such as lagging chromosomes, bi-nucleated, fragments and multipolor occurred but with very low frequencies in some treatments. X-rays treatments showed DNA alteration as with the appearance of polymorphic number of genetic bands.
[Khadijah, H. Alamoudi. **Evaluation of cytogenotoxic effects of X-rays on *Vicia faba* plant.** *J Am Sci* 2016;12(4):64-70]. ISSN 1545-1003 (print); ISSN 2375-7264 (online). http://www.jofamericanscience.org. 8. doi:10.7537/marsjas12041608.

Key words: Cytogenotoxic effects, mitotic division behavior, RAPD-RCR reaction.

1. Introduction

The ionizing radiation such as X-rays can be damaged any living tissue in the human body. The body attempts to repair the damage, but sometimes the damage is of a nature that cannot be repaired and these mistakes can lead to cancerous cells. Whereas, chromosomal damages can be used as a biomarker of possible effects of radiation exposure in hospital setting. The mean frequencies of different chromosomal aberrations were found to be higher in radiotherapy workers compared with the control[(1)]. Exposure of cells to ionizing radiation produces an extremely wide range of DNA damaging and mutational events. chromosomal aberrations and phenotypic mutation induced by types of ionizing radiation like: neutrons, gamma rays, X-rays, electron stream, protons and carbon ion beams in both plants and animals such as: lentil, tomato,, maize, barely, broad bean, wheat, rice, onion, *Trichosanthes anguina, Tradescantia*, *african Solanum incanum L*, mice, Lymphocytes and human hepatoma cells[(2-33)]. For evaluating genetic hazards of environmental mutagens, and /or carcinogens, Chromosomal aberrations induction and alteration of genetic material are the sensitive and important tests, because there is a clear association between chromosomal aberrations and certain types of cancer. The objective of this study is to evaluate the mutagenic effects of X-rays on *Vicia faba* root tip meristems by cytogenetic and molecular assays.

2. Materials and Methods

1- X- rays Treatments:

Vicia faba seeds (var. Giza 2) were exposed to different X- rays doses: 54, 108, 162 and 216 Kelo Volt (K.V.) by X- rays machine*)* Mode- IMS/ Energy 600 st *(*made in England, exposed to table top with 40 cm distance.

2- Cytological Analysis:

Treated and untreated *Vicia faba* seeds were germinated. *Vicia faba* root tips of 2-3 cm length were excised and fixed in Carnoy solution (3 ethanol: 1 acetic acid) for 24 h. and kept in refrigerator at 4 C in 70% ethanol until staining and examination. Aceto-carmine squash preparations were made and examined cytologically corroding to (Rank and Nielsen, 1993)[(34)]. From each treatment five preparations were examined to determine: mitotic index; different mitotic phases percentages; total abnormalities percentages; and different types of abnormalities frequencies abnormal cells frequencies in different mitotic phases.

3- RAPD- DNA Analysis:

Another groups of treated and untreated *Vicia faba* seeds were germinated in pots and left to grow. After 20 days from germination, treated and untreated *Vicia faba* leaves were taken to performed RAPD-PCR reaction. ***DNA Extraction*:** According to (Doyle and Doyle, 1990) [(35)], isolation of DNA from treated and untreated *Vicia faba* leaves, the Protocol for DNA isolation from leaves was taken

***Polymerase Chain Reaction (PCR)*:**

PCR reaction was conducted using Perkin Elmer (Germany) thermo cycler. RAPD was carried out using five random 10-mer primers (Operon Tech. Inc., USA) with the following sequences (5'→3') for RAPD analysis: OP-A20 (GTTGCGATCC)

OP-C11(AAAGCTGCGG)

OP-C16(CACACTCCAG)

OP-G17(ACGACCGACA)
OP-E18(GGACTGCAGA)

The reaction conditions were optimized and were mixtures consisted of the following: {dNTPs (2.5 mM)2.0 µl; Mg Cl2 (25 mM)1.5 µl; 10 x buffer 2.5 µl; primer (2.5 µM)2.0 µl; Template DNA (50 ng/ µl)20 µl; Taq (5 U/ µl)0.3 µl and ddH2O-14.7 µl}.The reaction mixtures were overlaid with a drop of light mineral oil per sample. Amplification was carried out in the thermo cycler programmed for 40 cycles as follows:{94oC/4 min(1 cycle); 94oC/1 min, 37oC/1 min, 75oC/2 min (38cycles); 72oC/12min(1cycle), 4oC(infinitive)}.

***Agarose gel electrophoresis*:** For resolving the RAPD-PCR products agarose (1.2%) was used. λ Phage DNA digested with *Bs*t EII was used as a standard DNA (15 fragments). Molecular sizes in K bp of the resulted fragments of the standard DNA ranged from 2.64 to 0.16.The run was performed for one hour at 100 V in Pharmacia submarine (20 cm X 20 cm). Bands were detected on UV- trans-illuminator and photographed by a Polaroid camera. Results were documented with Gel Doc 2000 (Bio RAD).

3. Results and Discussion

1- Cytological Studies:

The mitotic index from control preparations was found to be 5.85 in *Vicia faba* root tips (Table 1). A marked reduction in mitotic index was obtained after two X- rays treatments (54 and 216 K.V.) 4.87 and 4.91 respectively, and this reduction was attributed to the reduction of prophase stage index. On the other hand, mitotic index reduction did not affect by the increasing of X- rays dose. The inhibition of cell division was due to the inhibitory effect of X-rays on the inhibition of certain types of nuclear proteins which essential in the mitotic cycle. These results are in agreement with those of many researchers[2-30] after different ionizing radiation treatments such as: neutrons, gamma rays, X-rays, electron stream, protons and carbon ion beam in many plants such as: Lentil, tomato, broad bean, barely, maize,, wheat, onion, rice, and *Tradescantia.*

Pro- meta- ana and telo phases% recorded: 52.78%, 27.78% and 19.44% respectively in control, while different X- rays treatments caused unbalance of these percentages, whereas, pro- meta- ana and telo phases % were ranged from: (27.96% - 59.38%); (26.25% - 37.88%) and (14.38% - 36.49%) respectively in different X- rays treatments (Table, 1).

Increasing of the metaphase and (ana - telo) phases percentages in most treatments refer to the occurrence of abnormalities in these stages causing arrest at these stages. While, the reduction in the percentage of prophase stage in most treatments as resulted from reduction in cells number which inter to next mitotic division. Unbalanced mitotic stages percentages could be due to the X- rays effect on the biosynthesis of both nucleic acids (DNA or RNA) and nucleic proteins, which required for mitotic cycle and also different abnormalities, which occurred in different mitotic stages[3-31].

Abnormalities were observed at the different X-rays treatments and its percentage increased by the increasing of X-rays dose except for the 108 K.V. X-rays treatment (Table, 2). The greatest portion of this trait was observed in the meta and (ana - telo) phases, whereas abnormal pro- meta and (ana - telo) phases ranged from: (0.94% - 1.33%), (24.38%- 36.74%) and (13.75%- 33.18%), respectively after X- rays treatments, while they recorded 0.00%, 1.59% and 2.39% in control respectively. These results are in agreement with those of many researchers after different ionizing radiation treatments[5-20].

The most dominant abnormalities were: stickiness, C- metaphase, disturbed and bridges ranged from: (23.44% - 39.15%), (2.25% - 11.36%), (2.26% - 7.55%) and (1.56% - 6.76%) respectively (Table, 3). Stickiness% constitute about (60%- 70%) from total abnormalities and it increased by the increasing of X-rays dose in most treatments. Sticky chromosomes was shown in different mitotic stages (pro- meta and ana) phases (Fig. 1: a, b, c, e and g). Stickiness is regarded as a physiological effect exerted by X- rays in plant which affect on the proteins of chromosomes. Stickiness has been attributed to improper folding of chromosome fibers which makes of chromatids connected by means of subchromatid bridges and chromosome surface becomes sticky[37- 43].

Table (1): Effect of different X-rays treatments on mitotic Index, mitotic phases percentage in *Vicia faba* root tip cells.

X-rays Dose (K.V.)	Examined Cells No	Dividing Cells No.	Mitotic Index	Mitotic phases					
				Prophase		Metaphase		(Ana-telo) phase	
				No	%	No	%	No	%
Control	4309	252	5.85	133	52.78	70	27.78	49	19.44
54	4616	225	4.87	107	47..56	64	28.44	54	24
108	4795	320	6. 67	190	59. 38	84	26. 25	46	14.38
162	4903	264	5.38	99	37. 50	100	37.88	65	24.62
216	4293	211	4.91	59	27. 96	75	35. 55	77	36.49

K.V.: Kilo Volt

Table (2): The percentage of total abnormalities and abnormality mitotic phases produced by different X-rays treatments in *Vicia faba* root tip cells.

X-rays Dose (K.V.)	Divid Cells No.	Abnor-mal Cells No.	Total Abnomal Ities %	Mitotic phases								
				Prophase			Metaphase			(Ana-telo) phase		
				No.	Abn.	Abn. %	No.	Abn.	Abn. %	No.	Abn.	Abn. %
Control	252	10	3.97	133	0	0.00	74	4	1.59	55	6	2.39
54	225	119	52.89	107	3	1. 33	64	63	28	54	53	23.56
108	320	125	39.06	190	3	0. 94	84	78	24.38	46	44	13.75
162	264	162	61.36	100	3	1. 14	100	97	36.74	65	62	23.48
216	211	143	67.77	59	2	0. 95	75	71	33.65	77	70	33.18

K.V.: Kelo Volt

On the other hand, disturbance was shown in meta and ana phase (Fig. 1: d and e) as the partially action of X-rays on the spindle formation, therefore some chromosomes lost their ability to attach with the spindle fiber. The complete inhibition of X-rays on the spindle formation resulted at C- metaphase caused complete loss chromosomes of to their ability to continue to anaphase and arrested at metaphase (Fig. 1: f).

Chromosomal bridges were shown in (ana and telo) phases (Fig. 1: g and h), and may be attributed to the general stickiness of chromosomes and subsequent failure of anaphase separation and thus remain connected by bridges or they may be the result of chromosome breakage and reunion.

Other abnormalities such as Lagging chromosomes, bi-nucleated, fragment and multipolor occurred but with very low frequencies in some treatments (Table. 3) (Figs. 1: i, j, k and l). Occurrence of Laggards at metaphase may result from hindrance of prometaphase movement of the chromosomes accompanied by adhesion of centromeres to adjacent inner surface of plasma membrane, the Laggard were observed at metaphase failed to move properly toward poles and consequently, they appeared at the following stages.

Whoever, bi-nucleated cell appeared as the result of preceding telophase mitosis and failure of cell plate formation. On the other hand, multipolarity was presumably due to the splitting of the spindle fiber apparatus in three or more direction and accordingly the chromosome set was arranged haphazardly in three or more groups such as tripolor and tetrepolar[35-42].

In conclusion, it may be stated that the results presented are inconformity with previous finding of mutagenic effect of X- rays.

Table (3): Types and Proportions of abnormalities produced by different treatments of X-rays in *Vicia faba* root tip cells.

X-rays Dose (K. V.)	Control	54	108	162	216
Total abnormalities %	3.82	52.89	39.06	61.36	67.77
Stickiness	-	31.55	23.44	37. 88	39.15
C-metaphase	-	7.11	5.63	11.36	2.25
Disturbed	-	7.55	6.88	4.18	2.26
Bridge	2.29	4.44	1.56	4.17	6.76
multipolar	-	0.88	-	0.38	-
Lagging ch.	1.53	0.89	0.94	2.28	0.28
Bineiocleat	-	0.44	0.63	0.76	1.13
Fragments	-	-	-	0.38	0.56

K.V.: Kelo Volt

2-RAPD-PCR Analysis:

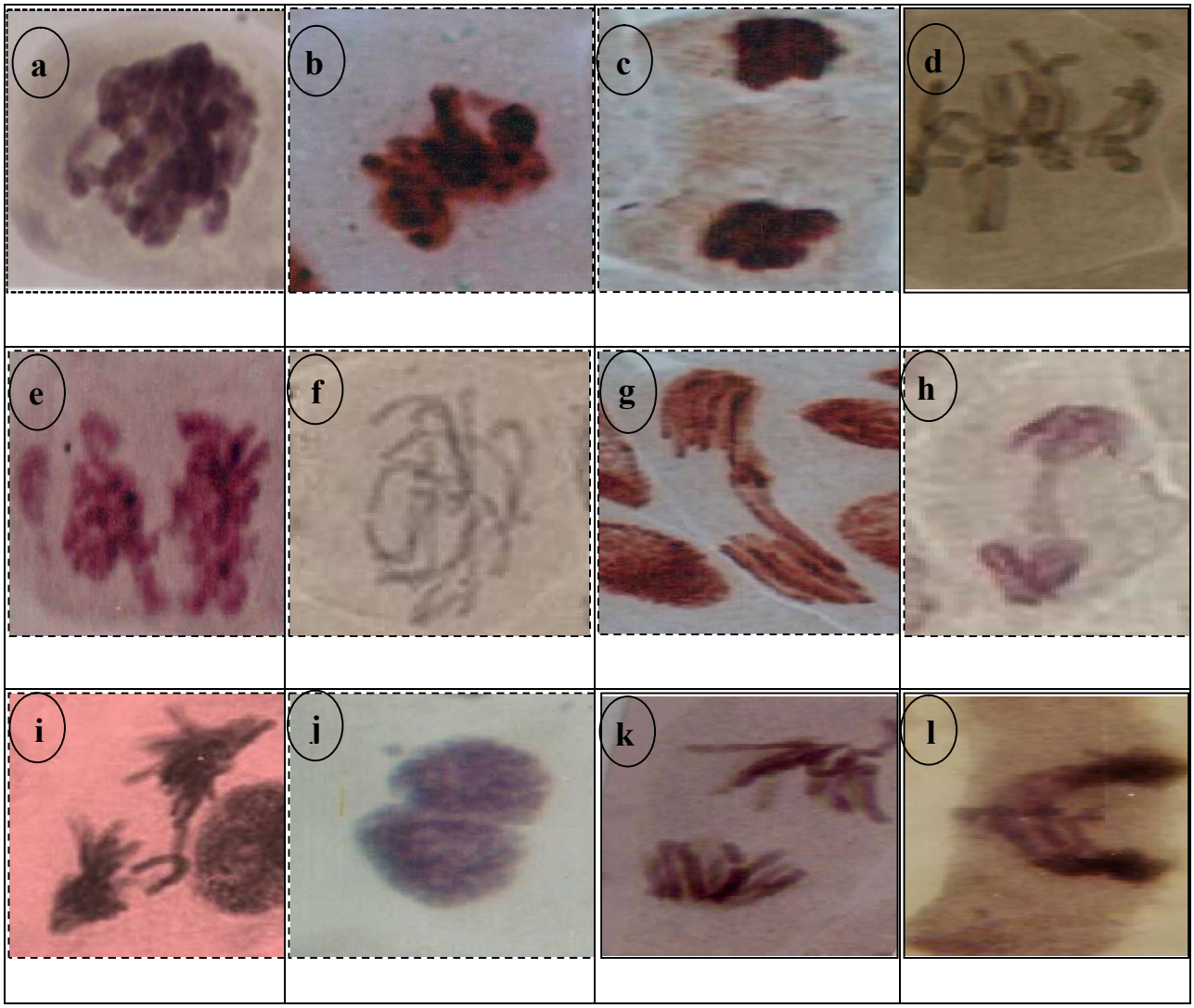
Genetic variation at the DNA level after X-rays treatments in *Vicia faba* was detected by RAPD analysis by using 5 primers (OP-A20, OP- C11, OP-C16, OP-G17and OP- E18). RAPD-PCR reaction by using four primers (OP- C11, OP-C16, OP-G17 and OP- E18) only revealed variation on DNA bands, whereas X-rays treatments altered the 11 DNA bands compared with the control, whereas 5 DNA bands disappearance and 6 new bands appearance. The polymorphic bands of the four primers were scored as present (1) and absent (0) as indicated in Table (4).

X-rays treatments caused disappearance of five DNA bands (1246, 1045, 455,419 and 324 b. p.) compared with the control. While, new sex DNA bands (236, 260, 275, 329, 347 and 367 b. p.) appearance after X-rays treatments compared with the control (Table 4, Fig.2).

Whereas, all X-rays treatments caused disappearance the two DNA bands: 1045 and 1246 b. p. (OP-C16). While 54 K.V. treatment only induced

two DNA bands: 329 and 347 b. p. (OP- E18). On the other hand, all X-rays treatments expect for 162 K.V. treatment caused disappearance the DNA band 419 b. p. (OP- E18), but 162 K.V. treatment only caused disappearance the DNA band 455 b. p. (OP- E18). Meanwhile, 108 K.V. and 162 K.V. treatments induced new DNA bands: 367 (OP- E18), 236 and 366 (OP-G17). On the other hand, 108 K.V. treatment caused disappearance the DNA band 324 b. p. (OP-G17), but the two X-rays treatments 54 K. V. and 162 K.V. induced new DNA band 275 b. p. (OP- C11) (Table 4, Fig.2). In conclusion RAPD-PCR method can be used as an investigational tool for X-rays induced genomic alterations. Furthermore, the present results suggest that RAPD-PDR fingerprinting together with cytological analysis can be a powerful strategy for assessing levels of X-rays exposure.

This observation gives good evidence to the ability of X-rays to induce insertion or mutations as a result of deletion compromises at least few nucleotides as revealed by the appearance or disappearance of many bands as compared with the control[38]. X-rays may generates free radicals which are interacted with DNA to account for the observed deletions as suggested by many workers in different plants after chemical or radiation treatments [34-45]. From cytological and molecular results, it could be concluded that X-rays have a cytogenotoxic effects.



Figure(1): Different mitotic abnormalities produced after different treatments of X-rays in *Vicia faba* root tip cells: a: stickiness in prophase; b: stickiness in metaphase; c: stickiness in anaphase; d: disturbed in metaphase; e: disturbed and stickiness in anaphase; f: C-metaphase; g: stickiness and bridge in anaphase; h: bridge in telophase; i: laggard in telophase; j: bi-nucleated; k: laggard and fragment in anaphase; l: multipolor.

Table (4): RAPD profile alterations in DNA bands as detected with 4-primers in *Vicia faba* plants after different X-rays treatments compared with the respective control

Primer Code	Sequences $5^{1} \rightarrow 3^{1}$	Size of polym. bands (b. p.)	Treatments				
			control	X-rays doses (K.V.)			
				54	108	162	216
OP-E18	5-GGACTGCAGA-3	455	1	1	1	0	1
		419	1	0	0	1	0
		367	0	0	1	0	1
		347	0	1	0	0	0
		329	0	1	0	0	0
OP-G17	5-ACGACCGACA-3	324	1	1	0	1	1
		260	0	0	0	0	1
		236	0	0	1	0	0
OP-C16	5-CACACTCCAG-3	1246	1	0	0	0	0
		1045	1	0	0	0	0
OP-C11	5-AAAGCTGCGG-3	275	0	1	0	1	0

1: Appearance of bands. 0: Disappearance of bands.

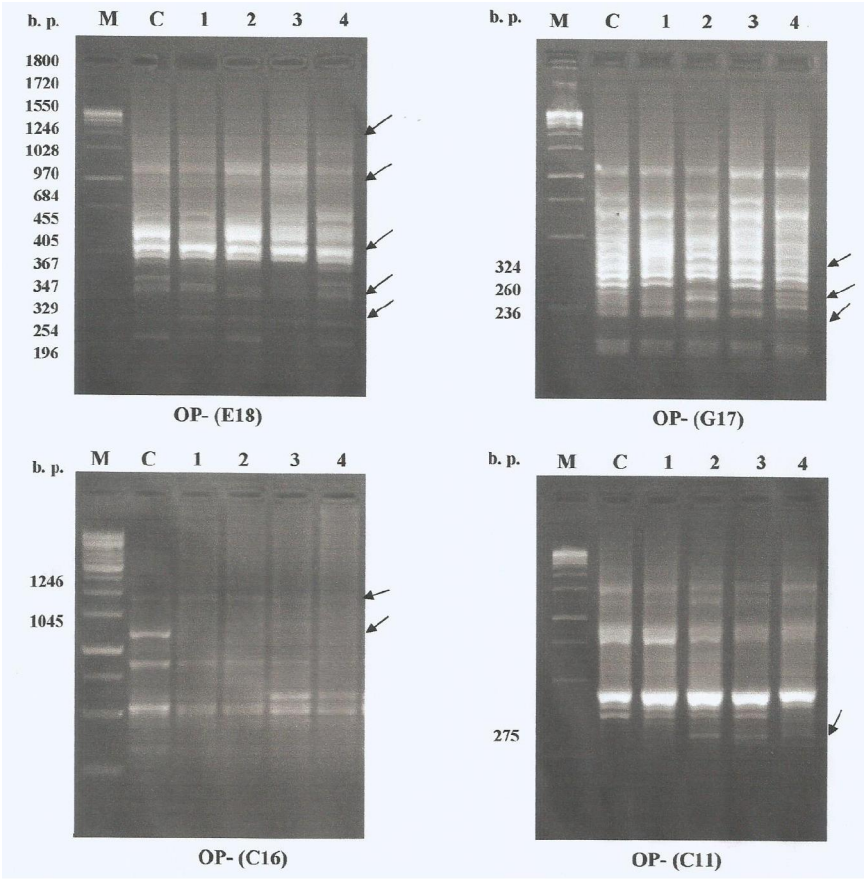


Fig.(2): RAPD profiles of genomic DNA of *Vicia faba* plants after different X-rays treatments by using four primers. (M: DNA marker, C: control, (1, 2, 3, 4): X-rays treatments with doses: 54, 108, 162 and 216 K. V. respectively)

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3/12/2016