Mutagenic effect of X-rays on *Vicia faba* plant

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Abstract: The mutagenic effect of different x-rays doses were examined on *Vicia faba* plant by measuring some growth traits, photosynthetic pigments and changes in DNA using RAPD (PCR) analysis. X-rays treatments caused a significant reduction in plant height, plant fresh weight and both chlorophyll (a) and (b) contents. Consequently, there is in strong correlation between the DNA alterations as shown a polymorphic number of genetic bands using RAPD_PCR products comparing with control. Results strongly suggest that x-rays have a mutagenic effect on *Vicia faba* plant.
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Key words: *X-rays,mutation,RAPD (PCR) analysis, DNA alterations*

1. Introduction

X-rays cause chromosomal damages in radio therapy workers due to radiation exposure action in hospital setting, Movafagh et al., (2007).

Any living tissue in the human body can be damaged by the ionizing radiation such as x-rays. The body attempts to repair the damage, but sometimes the damage is of a nature that cannot be repaired or it is too severe or wide spread to be repaired. Also mistakes made in the natural repair process can lead to cancerous cells. In general, the amount and duration of radiation exposure effects the severity or type of health effect, Podgorsak (2005) There are two broad categories of health effects: stochastic and non-stochastic. Stochastic effects are associated with long-term, low level (chronic) exposure of radiation. This type of exposure to radiation can cause changes in DNA (mutation), the "blue prints" that ensure cell repair and replacement produce a perfect copy of the original cell. Cancer is considered the primary stochastic health effect from radiation exposure, Kocher and Trabalka (2000)

Non-stochastic effects appear exposed cases to high- Levels of radiation and become more severe as the exposure increase. Short-term high-level exposure is referred to as "acute" exposure. Unlike cancer, health effects from "acute" exposure to radiation usually appear quickly. Acute health effects include burns and radiation sickness which is manifested in nausea, weakness, hair Loss, skin burns or diminished organ function, Ghiassi et al.,(2002).

All types of ionizing radiation such as: neutrons, gamma rays, x-rays, electron stream, protons and carbon ion beams induced chromosomal aberration, DNA damages and phenotypic mutation many organisms in both plant and animal such as: tomato, lentil, maize, broad bean, barely, wheat, rice, onion, Tradescantia, Trichosan thes anguina, *African solanum incanum L*, mice, human hepatoma cell and lymphocytes, Thappa and Menon (1973); Geard (1983): Gramatikova (1989); Maillie et al., (1992); Ofuchi et al., (1999); Ishihar et al., (2000); Pavicic(2004); Ikeda(2007).

The purpose of this study is evaluation of the mutagenic effect of x-rays on *Vicia faba* plants and measuring of some growth traits, photosynthetic pigments and screening DNA alteration using Random Amplified Polymorphic DNA assay method (RAPD-PCR).

2. Materials and Methods:

2.1. Materials:

2.1.1.Sample of the study:

Seeds of *Vicia faba* c.v. Giza 402 were used and they were obtained from the Agricutural Research Centre, Giza, Egypt. Air dried seeds exposed to different x-rays doses: 54,108,162 and 216 kelo volt (k.v.) by x-rays machine: Mode-IMS/ Energy 600 st- exposed on table top with 40 cm distance, (made in England).

2.2.Methods:

2.2.1.Measurements of some growth traits and photosynthetic pigments:

Treated and un-treaded *Vicia Faba* seeds were sown in pots in the green house in split plot design experiment with ten plants in three replications. After 21 days, plants height(cm) and fresh weights per plant (g) were measured, pigments (chl.a, chl.b and carotenoids were determined spectro photometrially (Lichtenthaler,1987).

- ***Statistical analysis*** for the experimental values of using t-tests was performed.
- ***Inhibitory rates (%)*** of the obvious traits were calculated using the following formula:

$$IR= (1 _ \frac{X}{Y})x100$$

Where: **X:** the average of control trait.
Y: the average of the same trait in treated *Vicia faba*.

2.2.2.RAPD – PCR Analysis

DNA Extraction:

Isolation of DNA was performed from treated and untreated *Vicia faba* leaves after 21 days from sowning. DNA isolation was carried out by the CTAB, Doyle and Doyle (1990) withmodifications.

Five grams fresh weight were ground to fine powder in liquid nitrogen in a pre-chilled mortar, mixed in 5 ml pre-heated CTAB buffer, and incubated at 60°C for 30 min. with occasional shaking. DNA was extracted in chloroform-isoamyl alcohol (24:1) precipitated in cold isopropanol, washed in 70% ethanol, 10 mM ammonium acetate and suspended in 0.5ml TE buffer. The solution was treated with 50mg/ml RNase A (Bochringer Manheim, Germany) for 30 min. at 37°C and treated with 50mg/ml proteinase K (Bochringer Manheim, Germany) for 30min. at 42°C followed by comparison to serial dilutions of Lambda-DNA, electrophoresis in 0.8% agarose gel, stained in 0.2mg/ml ethidium bromide and photographed under UV illumination.

***DNA Amplification:**

Amplification was performed in 10mg reaction mix containing 20ng genomic DNA, 0.5 unit Tag polymerase (Promega, USA), 200µg each of d ATP, d CTP, d GTA and d TTP, 5 p mole random primer (OperonTech.Inc.,USA) and appropriate amplification buffer

Table(1):Primer sequences

Primer name	Sequences 5'→ 3'
Op - B16	5'-TTTGCCCGGA-3'
Op - E20	5'-AACGGTGACC-3'
Op - G3	5'-GAGCCCTCCA-3'
Op - O16	5'-TCGGCGGTTC-3'
Op - B3	5'-CATCCCCCTG-3'

The mixture was assembled on ice, overlaid with a drop of mineral oil. Amplification was performed for 45 cycles, using UNO thermal cycler (Biometra, Germany) as follows: One cycle at 92C° for 3 min, 45 cycles at 92 °C for 30 sec., 35 °C for 60 sec and 72 °C for 2 min. The reaction was finally incubated at 72 °C for 10 min. and further 10 min. at 63 °C. Electrophoresis was done in 2% agarose gel (1% Nusieve GTG, 1% Seakam L.E., FMC Bioproducts) in TAE buffer (0.04 M Tris – acetate, 1 mM EDTA, pH8).RAPD products were stained in 0.2 µg /ml ethidium bromide and photographed under UV-light. Results were documented with Gel Doc 2000 (Bio RAD).

3. Results and Discussion:

3.1-Growth Traits and Photosynthetic Pigments:-

Plant height inhibition rates in *Vicia faba* plants after different X-rays treatments are shown in fig.(1).It revealed a significance decrease in this trait only in 162 K.V. X-rays treatment(-12%).

On the other hand, significant decrease was observed in inhibition rates of *Vicia faba* fresh weight in all X-rays treatments except for 54 K.V. X-rays treatments. While 162 K.V. X-rays treatment was recorded highly significant in this trait (-59%) (Fig.2).

Inhibitory rate of chlorophyll (a), chlorophyll (b) and carotenoids contents in *Vicia faba* plants after X-rays treatments are shown in Fig.(1), (2) and (3). Significant decrease was observed in inhibitory rate for both two traits: chlorophyll (a) and (b) in all X-rays treatments except for 54 K.V. X-rays treatment, While 162 K.V. X-rays was recorded highly significant in the both Last traits (-84% and -75%).

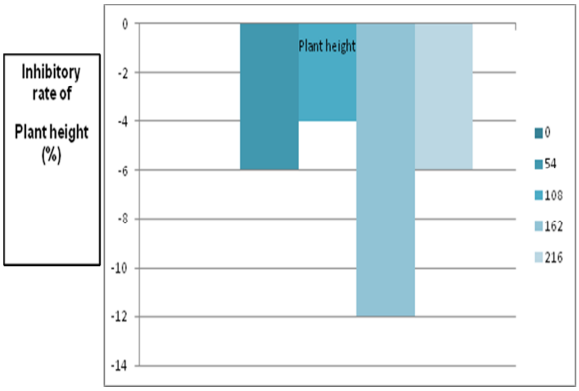
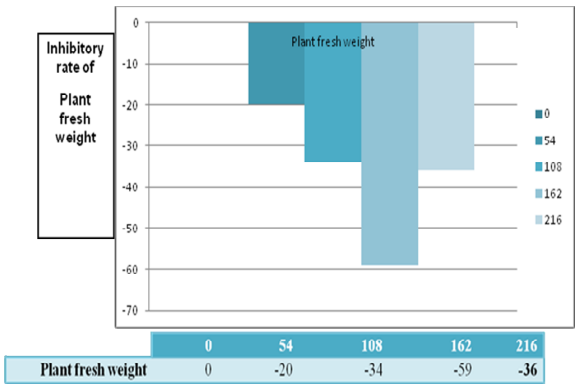


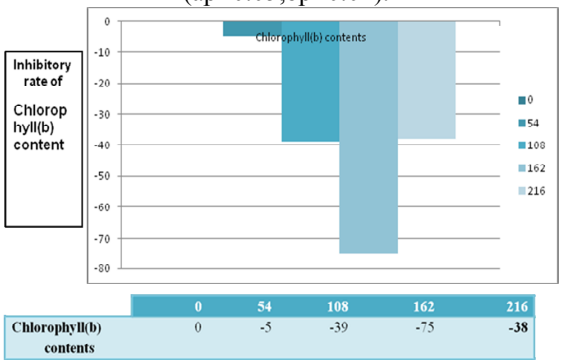
Fig1: Plant height inhibition rates in *Vicia faba* plants after different x-rays treatments. (ap < 0.05)



	0	54	108	162	216
Plant fresh weight	0	-20	-34	-59	-36

X- rays doses (k-v.)

Fig2: plant fresh weight inhibitions rates in *Vicia faba* plants after different x-rays treatments. (ap<0.05,bp<0.01).



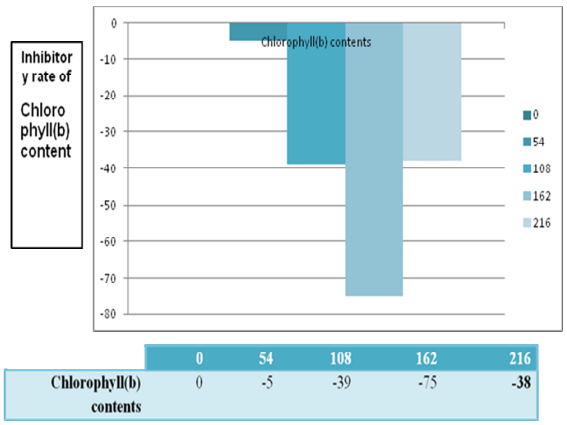
	0	54	108	162	216
Chlorophyll(b) contents	0	-5	-39	-75	-38

X- rays doses (k-v.)

Fig3: chloroplyll(a) contents inhibitions rates in *Vicia faba* plants after x-rays treatments. (ap<0.05,bp<0.01).

Whereas, all X-rays treatment didn't show any significant in carotenoids contents in *Vicis faba* plant (Fig.5). The present investigation revealed the mutagenic potential of photosynthetic pigments reduction in both chlorophyll (a) and (b) contents and thus consequently due to decreasing of *Vicia faba* growth rate 9 plant height and plant fresh weight). Reduction of photosynthetic pigments (chlorophyll a and b) in *Vicia faba* plant after X-rays action on the genes which control of the production of these traits.

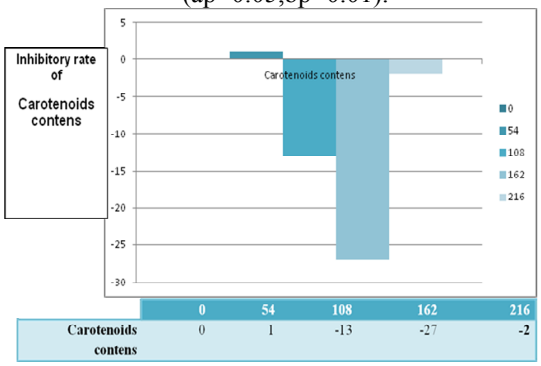
The obtained results are in agreement with many researchers in different plants after chemical or radiation treatments, Thappa and Menon (1973); Hassan et al, (1988); Yi-ping et al.,(2005); Grant and Owens (2006); Qian et al.,(2006); Ikeda et al.,(2007); Srivastavaand Singh (2009)



	0	54	108	162	216
Chlorophyll(b) contents	0	-5	-39	-75	-38

X- rays doses (k-v.)

Fig4: chloroplyll(b) contents inhibitions rates in *Vicia faba* plants after x-rays treatments. (ap<0.05,bp<0.01).



	0	54	108	162	216
Carotenoids contens	0	1	-13	-27	-2

X- rays doses (k-v.)

Fig5: Carotenoids contents inhibitions rates in *Vicia faba* plants after x-rays treatments. (ap<0.05,bp<0.01).

3.2-RAPD – Profile:

Genetic variation at the DNA level after X-rays treatments in *Vicia faba* plant was detected by RAPD analysis. The polymorphic bands of four primers were scored as present (+) and absent (-) as indicated in Table (1). OP_B3 primer produced four polymorphic bands (278,477,891 and 998 b.p.) after X-rays treatments compared with the control (Fig. 6, Table 1). On the other hand, two primers i:e: OP_G3 and OP_O16 gave two polymorphic bands with size (394,344 b.p.) and (1339,700 b.p) respectively after X-rays treatments. While, both the two primer i:e: OP_B16 and OP_E20 induced one polymorphic band with size 187 and 686 b.p. respectively after X-rays treatments compared with the control (Table 1, Fig.6).

X-rays treatments caused disappearance of five DNA bands with size of (187,1339,700,998 and 278 b.p.) compared with the control, While these treatments due to the presence of new five DNA

bands with size of (686, 394, 344, 981, 477 b.p.) which was absent in the control (Table 1.,Fig. 6).

RAPD-PCR based assays are important as a genome wide DNA variation screening strategy. Toxicant induced genotoxic effects, DNA variation, DNA damage, genetic instability and mutagenic effects have been evaluated with RAPD analysis successfully by pervious work. RAPD assay has proved useful to detect genomic instability manifested such as point mutation, genetic,chromosomal rearrangements, deletion and insertions,Baeshin et al., (2009). RAPD is likely to detect genomic instability as the newly growing and developing cells will produce a clone of dividing daughter cells. Thus, the proportion of cells presenting the same genomic instability is high and easy to detect. In the field of genetic toxicology most RAPD studies describe changes such as differences in band intensity, as well as, gain/loss of RAPD bands, defined as diagnostic RAPD (Guzin et al., 2010).

In conclusion RAPD-PRC method can be used as an investigation tool for X-rays induced genomic alterations. Furthermore, the present results suggest that RAPD_PCR finger printing together with physiological parameter can be a powerful strategy for assessing levels of X -rays exposure. OP_B3 primer was informative for detecting X -rays induced specific genomic alterations. From the obvious results we concluded that x-rays have a mutagenic effect in *Vicia faba* plant.

Table(1): RAPD profile alteration in DNA bands as detected with five primer in *Vicia faba* plants after different x-rays treatments.

primer	Sequences 5'→ 3'	Siz of polym.bands (b.p.)	Treatments				
			control	X-rays doses (k.v.)			
				54	108	162	216
B16	5'-TTTGCCCGGA-3'	187	+	+	-	-	+
E20	5'-AACGGTGACC-3'	686	-	-	+	+	-
G3	5'-GAGCCCTCCA-3'	394	-	-	+	+	+
		344	-	+	-	-	-
O16	5'-TCGGCGGTTC-3'	1339	+	+	+	-	+
		700	+	+	+	-	+
B3	5'-CATCCCCCTG-3'	998	+	-	-	-	-
		891	-	-	-	+	+
		477	-	-	-	-	+
		278	+	+	-	+	+

+ appearance of bands.

-Disappearance of bands.

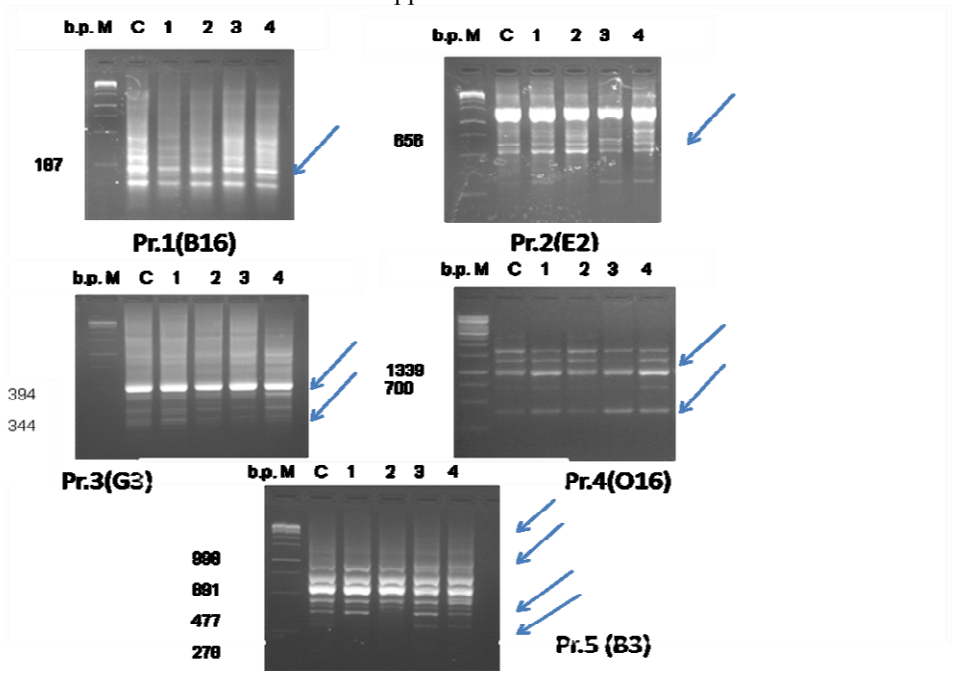


Fig.(6): RAPD profiles of genomic DNA of *Vicia faba* plants after different x-rays treatments by using 5 primer.
(*M: DNA marker, C:control, (1,2,3,4): x-rays treatments with doses:54,108,162,and 216 k.v. respectively.*

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