

CYTOLOGICAL EFFECT OF GAMMA IRRADIATION ON MITOTIC ACTIVITY ON VICIA FABA PLANTS.

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ABSTRACT

The present study was carried to investigate the cytological effects of six doses of gamma rays (1,2,3,4,5 K.rad and fractionated doses (1 K.rad/24hrs for 5 days) in root meristems of *Vicia faba* plant. The data of the present study revealed that all radiation treatments reduced mitotic index and increased total chromosomal abnormalities. The most common of them were: stickness, laggards, bridges, micronuclei, multinuclei, disturbed, c-metaphase, splitprophase, chromosome ring, tripolar and tetrapolar. The results also illustrated that there was a negative correlation between radiation dose and mitotic index but there was a positive correlation between radiation dose and total chromosomal abnormalities. Fractionated dose (1 K.rad/24hrs for 5 days) had effects on mitotic index and total chromosomal abnormalities less than dose 3, 4 and 5 K.rad but it had intermediate effects between two dose 2 and 3 K.rad.

INTRODUCTION

Using gamma irradiation, protons and ion beam caused different chromosomal aberration in different plants (Jayabalan and Rao, 1983; Lu-ting and Xie liqing, 1991; El-Sayed *et al.* 1994; Li-Guoquan *et al.* 1996; Wang-Cailian *et al.* 1998). The induction of micronuclei demonstrated nonlinear kinetics with a significant increase over controls above 200 rads gamma rays, 10 m M EMS or 250 M ENU on maize root tip cells (Wanger and Plewa, 1985.) (Olejniczak, 1986) showed that the combined dose of SA + MNUA and MNUA + gamma rays caused marked increase of chromosomal aberrations number in maize root tips as compared to separately acting mutagens. Rizzoni *et al.* (1987) found that 1.384 R of x-rays caused the micronucleus frequency twice than the control in *Vicia faba* root tips. Formation of chromosomal aberration and micronuclei was effectively stimulated by low radiation doses in *Vicia faba* root tips (Kuglik *et al.* 1990). Ye-Abao (1991) found that electron stream caused chromosomal aberration in bean and barley such as: chromosome bridge, fragment, ring chromosome and micronuclei. He also found a positive linear correlation between the micronuclei rate in root tip cells and the aberration rate. There was a negative correlation between radiation dose and mitotic index and a positive correlation between radiation dose and mitotic abnormalities. (Datta *et al.* 1991; Al-Safady and Simon 1992; Wang-Cailian *et al.* 1995; Yi-Huying *et al.* 1995; Hu-Baomin *et al.* 1996 and Wang-Cailian *et al.* 1998). The effect of ion beam were lower on chromosomal aberration as compared with gamma rays in root tip stevia cells. (Wang-Cailian *et al.* 1998).

MATERIALS AND METHODS

Vicia faba seeds (cv. Giza 2) were irradiated with 1,2,3,4,5 k rad and fractionated dose (1k rad/24 hrs. for 5 days) using the facilities at the National Center for Research and Radiation Technology, Cairo, Egypt. The dose rate was 7.5 rad/sec. Treated and untreated seeds were germinated. Root tips were cut off and fixed in Carnoy's solution (6 ethyl alcohol +3 chloroform +1 acetic acid glacial) and investigated with usual acetocarmine squash technique, Brown (1949). Five preparation from each treatment were examined to determine the:-

1. Mitotic Index (M.I.)= (No. of divided cells/Total No. of examined cells)%.
2. %total of cells abnormalities = (No. of cells abnormalities/No. of divided cells)%.
3. %of each abnormality=(No. of this abnormality/No. of cells abnormalities)%

RESULTS AND DISCUSSION

I. Mitotic Index (M.I.).

The mitotic index from control preparations was found to be 8.21 in *Vicia faba* root tips (Table.1). In *Vicia faba* root tips, a considerable decrease in mitotic index was noticed the following treatments with different doses of gamma rays. (Table.1). There were a negative linear correlation between radiation doses and mitotic index from 1 to 5 K rad doses but fractionated doses (1K rad /24hrs for 5days) had intermediate effect between two doses 2 and 3 K rad.(Fig.1).The inhibition of cell division could be the effect of gamma rays on protein synthesis (Al-Safady and Simon 1992) showed that gamma rays induced a reduction in mitotic index , they attributed such reduction to the inhibition of certain types of nuclear proteins essential in the mitotic cycle.

Table (1): Mitotic index in *Vicia faba* root tips after seed irradiation.

Treatments	No. of cells exam.	No. of dividing cells	Mitotic Index
Control	3300	271	8.21
1K rad	3460	211	6.10
2K rad	4250	245	5.76
3K rad	3010	150	4.98
4K rad	5910	241	4.08
5K rad	5370	160	2.98
Fractionated dose (1K rad/24hrs for 5days)	4345	220	5.06

II. Mitotic Abnormalities

The total percentage of chromosomal abnormalities in *Vicia faba* root tips ranged from 15.17 to 75.63 % after seed irradiation. (Table .2). There was a positive linear correlation between radiation doses from 1to5 K rad and chromosomal abnormalities % but fractionated dose (1K rad/24 hrs for 5 days) had intermediate effect between two doses 2and3 K rad. (Fig.2) This conclusion was in harmony with Datta *et.al* .1991, Al Safady and Simon

(1992).It could be noticed from table.2 that gamma rays induced a wide rang of mitotic abnormalities including: split prophase , C-metaphase, stickness,

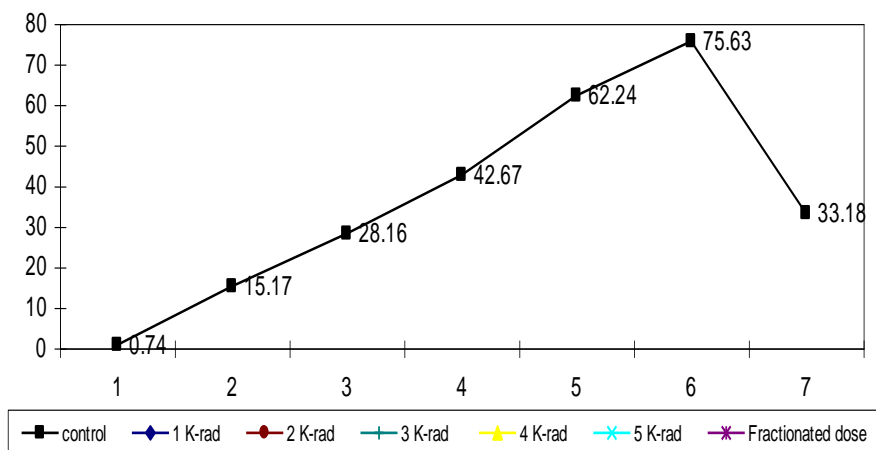


Fig.1: Mitotic Index in *Vicia faba* root tips after seed irradiation.

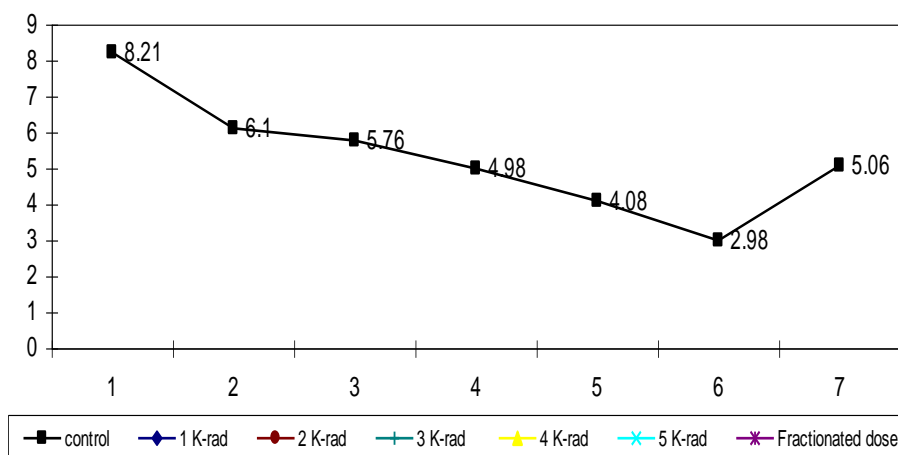


Fig.2: Percentage of total chromosomal abnormalities in *Vicia faba* root tips after seed irradiation.

breaks, fragments, lagging ch., bridges, disturbed ch., Chromosome ring, tripolar, tetrapolar, micronuclei and multinuclei (Fig. from 3 to 18). The percentage of stickiness, breaks and fragments, (micro-multi) nuclei were

Fig. (3): Split prophase

Fig. (4): C - Metaphse

Fig. (5): Metaphase stickiness

Fig. (6): Anaphase stickiness

Fig. (7): Metapjase stickiness with lagging chromosome

Fig. (8): Metaphse stickiness with fragment

Fig. (9): Metaphase stickiness with ring chromosome

Fig. (10): Anaphase stickiness with fragment, lagging, chromosome bridge.

Fig. (11): Early telophase with chromosome bridge

Fig. (12): Telophase with fragment, lagging and chromosome bridge

Fig. (13): Disturbed Chromosome

Fig. (14): Ring chromosome

Fig. (15): Tripolar

Fig. (16): Tetrapolar

Fig. (17): Micronuclei

Fig. (18): Multinuclei.

more as compared to other types of observed abnormalities. The most dominant type of observed abnormalities was chromosome stickiness. Its percentage increased with increasing of radiation dose and ranged from 37.50 to 80.17% (Table.2). On the other hand, micro and multinuclei stimulated and increased by low radiation dose from 1 to 3 K rad (with range 13.25 to 25%), but it decreased by high radiation dose (4 and 5 K rad) with range 15 and 7.44% (Table.2). These findings were in harmony with Kuglik *et al.* 1990. Breaks and fragments chromosome percentage didn't effect with radiation dose. (Table.2). Disturbed chromosome and bridges percentage were ranged from (3.13-11.50%) and (1.56-8.28%) respectively and they increased with the increasing of radiation dose, on the other hand lagging chromosome percentage generally decreased by increase of radiation dose and ranged from 5.48 to 15.63%. (Table.2). Meanwhile, C-metaphase was observed with low percentage in all radiation treatments, but split prophase, chromosome ring tripolar and tetrapolar were observed only in the highest dose (5K rad) with low percentage. Fractionated dose (1K rad/24hrs for 5days) recorded generally intermediate percentage in different chromosomal abnormalities between those obtained in the two dose 2 and 3K rad. (Table.2). The types of chromosomal abnormalities by gamma rays may be grouped in the following classes. The first comprises those which are due to an action of radiation on spindle apparatus such as: C-metaphase, disturbed chromosome multipolar (tripolar and tetrapolar) and lagging chromosome. The inhibition of spindle apparatus by gamma rays may be to its effect on proteins constituting the spindle apparatus. (Hu-Baomin *et al.* 1996. and Wang-Caillan *et al.* 1998). The second class of chromosomal abnormalities produced by radiation was chromosomal abnormalities comprised of chromosome such as: breaks, fragments and bridges. (Al-Safady and Simon 1992, Yi-Huying *et al.* 1995 and Hu-Baomin *et al.* 1996). Stickiness is regarded as a physiological effect exerted by gamma rays in plant which has been considered to effect the proteins of chromosomes. Stickiness has been attributed to improper folding of chromosome fibers which makes of chromatids connected by means of subchromatid bridges (Datta *et al.* 1991, El-Sayed *et al.* 1994 and Wang-Caillan *et al.* 1998). Some abnormal interphase cells have been recorded following various radiation doses. The abnormalities observed were the formation of micro and multinuclei. The formation of multinuclei may be the result of preceding multipolar mitosis or the failure of cell plate formation. Wang-caillan *et al.* (1995). On the other hand, micronuclei may be the result of lagging chromosome or chromosome fragments produced during preceding division. Hu-Baomin *et al.* (1996) and Wang –Caillan *et al.* (1998).

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**التأثير السيتولوجي لأشعة جاما على كفاءة الانقسام الميتوزي في نبات الفول.
أسمهان أحمد محمود
المركز القومي لبحوث و تكنولوجيا الأشعاع ص.ب. ٢٩ مدينة نصر القاهرة.**

- تناول هذا البحث دراسة تأثير ٦ جرعات لأشعة جاما (١, ٢, ٣, ٤, ٥ كيلو راد) والجرعة الأشعاعية المجزئة ٢٤ راد/ ساعة لمدة خمسة ايام على كفاءة الانقسام الميتوزي لنبات الفول (جيزة ٢) وكانت النتائج كالآتي:-
١. أدت المعاملات الأشعاعية إلى انخفاض في معدل الانقسام الميتوزي (M.I.) بينما أدت تلك المعاملات لارتفاع النسبة الكلية للتغيرات الكروموسومية.
 ٢. أدت المعاملات الأشعاعية لظهور عديد من طرز التغيرات الكروموسومية منها: لزوجة الكروموسومات – كروموسومات متلكنة – كروموسومات مشتتة – كبارى كروموسومية – نويات – ثلاثية الأقطاب – رباعية الأقطاب .
 ٣. وجد أن هناك علاقة سالبة بين زيادة الجرعة الأشعاعية و معدل الانقسام الميتوزي (M.I.) بينما توجد علاقة موجبة بين زيادة الجرعة الأشعاعية و النسبة الكلية للتغيرات الكروموسومية.
 ٤. كانت الجرعة الأشعاعية المجزئة ١٤ راد/ ٢٤ ساعة لمدة خمسة أيام أقل تأثيراً من الجرعات ٥, ٣, ٤ ك راد على معدل الانقسام الميتوزي (M.I.) و النسبة الكلية للتغيرات الكروموسومية و لكنها كانت ذات تأثير وسطى بين الجرعتين ٢, ٣ ك راد.

Table (2): Percentage of abnormalities in mitotic stages of Vicia faba root tips after seed irradiation.

Treatments	Split prophase	C-Meta-phase	Stickiness	Breaks and Fragments	Lagging Ch.	Bridges	Disturbed Ch.	Chromosome Ring	Tripolar	Tetrapolar	(Micro and Multi) Nuclei	%Total abnormalites
Control	-	-	0.37	-	-	-	0.37	-	-	-	-	0.74
1 K-rad	-	3.13	37.50	15.63	15.63	-	3.13	-	-	-	25.00	15.17
2 K-rad	-	1.44	39.13	14.49	10.14	2.99	4.26	-	-	-	27.54	28.16
3 K-rad	-	1.56	51.56	15.63	9.38	1.56	6.25	-	-	-	31.25	42.67
4 K-rad	-	2.67	65.33	16.67	8.00	2.67	8.00	-	-	-	15.00	62.24
5 K-rad	0.83	4.43	80.17	15.70	6.61	8.26	11.57	1.65	3.31	2.45	7.44	75.63
Fractionated Dose	-	2.74	41.10	15.07	5.48	2.74	5.48	-	-	-	27.40	33.18
Over all Mean	0.12	2.28	45.02	13.31	7.89	2.60	5.58	0.24	0.47	0.35	19.09	36.83