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GAMMA RAY - INDUCED MITOTIC ABNORMALITIES IN *Helianthus annuus* L. VARIETY EKIZ 1

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SUMMARY

In this study, mitotic effects of gamma rays on Ekiz 1 variety belonging to *Helianthus annuus* L. (2n = 34) in the M_0 (first irradiated seeds), M_1 and M_2 generations have been investigated. Seeds (M_0) were irradiated with gamma rays at 10, 20, 30, 40 and 50 kR doses. Percentage of total abnormalities in the M_0, M_1 and M_2 generations increased parallel to the increasing dose of radiation. These abnormalites have been observed as C-metaphase, chromosome stickiness, laggards and bridges with or without fragment. Mitotic index (M.I.) in the M_0, M_1 and M_2 generations has decreased parallel to the dose increase. When the generations are compared, both the amounts of decrease in mitotic index and in the percentage of mitotic abnormalities were mostly observed in M_0.

Key words: ***Helianthus annuus* L., mitotic index, gamma rays, chromosome aberrations**

INTRODUCTION

The effect of radioactivity on the environment varies depending on the strength and duration of radiation and the type of rays. Nuclear experiments and accidents cause the whole ecosphere to become polluted. In the biological system, the most commonly used plant part, *i.e.*, the seed, suffers the most extensive damage due to radiation. Cytogenetic effects of ionizing radiation on different plants have been studied by several authors such as Sax (1941), Saporrow *et al.* (1952), Caldecott *et al.* (1954), Evans (1962), Tarar and Dynansagar (1983), Savaþkan and Toker (1991).

Although there are many reports on *Helianthus annuus* L., which is a significant plant in terms of oil, the studies on mitotic abnormalities caused by gamma

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radiation have not been very extensive. In this study, the effects of gamma rays on Ekiz 1 variety of *Helianthus annuus* L. in the M_0 (first irradiated seeds), M_1 and M_2 generations have been investigated. Ekiz 1 is a product of a study aimed at obtaining a new variety of *Helianthus annuus* L. that has a high yield of fat in seed and is resistant to *Orobanche cumana* in the conditions of Turkey.

MATERIAL AND METHODS

The seeds of *Helianthus annuus* L. variety Ekiz 1 have been treated with ^{60}Co sources at the doses of 10, 20, 30, 40 and 50 kR (seed moisture relativity 5.68%). These seeds have been germinated in germination plates placed in an incubator at 22°C. Healty root tips have been fixed in Carnoy's (3:1, ethanol: acetic acid) for 24 hours. Later, they have been hydrolized with 1 N HC1 at 60°C for 12 minutes and stained with 1% asetocarmine. For each dose, at least 10 permanent preparations have been prepared with the squash method. The same procedure has been repeated for the M_1 and M_2 generations. The data from areas which showed good separation in the preparations have been evaluated by means of the t- test:

$$t = \frac{P_0 - P_1}{\sqrt{\frac{P_0 \cdot (100 - P_0)}{n_0 - 1} + \frac{P_1 \cdot (100 - P_1)}{n_1 - 1}}}$$

RESULTS

Mitotic index (M.I.), total mitotic abnormality percentage and mitotic abnormality types of the M_0, M_1 and M_2 generations are given in Table 1 and Table 2. Abnormality types have been characterized as C-metaphase (Figure 1a), chromosome stickness (Figure 1b), laggards at metaphase (Figure 1c) and anaphase (Figure 1d, e, f), chromosome and chromatine bridges with or without fragment at anaphase and telophase (Figure 1g, h, k, 1). Some of the bridges were sticky (Figure 1j).

Decreases in the mitotic index of the M_0, M_1 and M_2 generations of *Helianthus annuus* L. occurred simultaneously with increases in the radiation dose. The decrease in the mitotic index was significant at 1% only for 40 and 50 kR in the M_2 generation whereas it was significant at 1% for all of the doses in the M_0 and M_1 generations (Table 1).

Total abnormality percentage in the M_0, M_1 and M_2 generations increased simultaneously with the increasing dose of radiation (Table 2). This increase was significant at 1% for all doses. The total abnormality percantage observed in the M_1 and M_2 generations was lower than that observed in M_0.

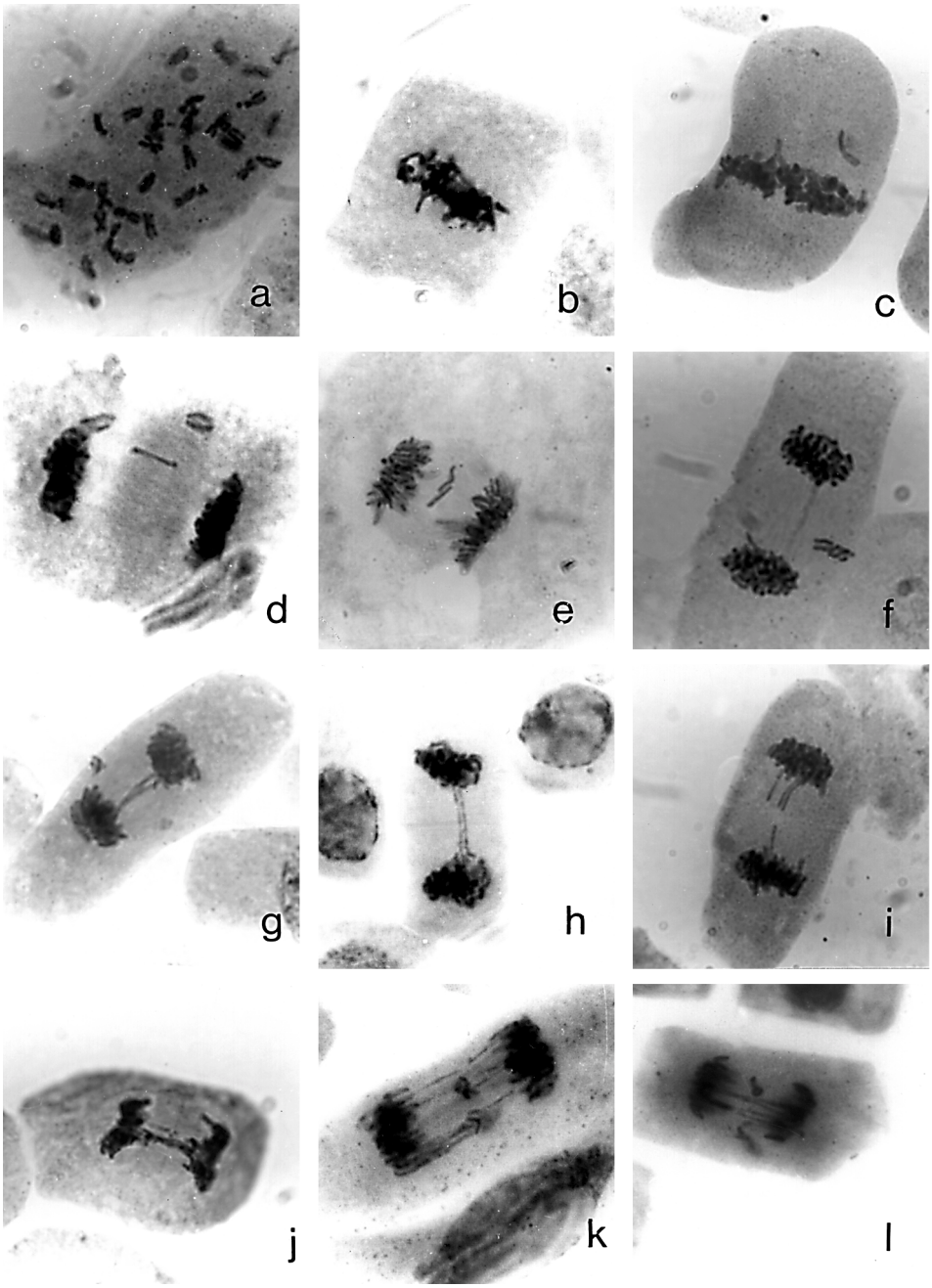

Figure 1: (a) c-metaphase; (b) stickiness; (c) stickiness and lagging chromosome; (d, e, f) laggards at anaphase; (g, h) double bridges at anaphase; (i) broken bridges at anaphase; (j) sticky bridge; (k, l) bridges with fragments and chromosome (x 1100)

Table 1: Percentage of cells showing mitotic division and mitotic abnormalities in root tips of the M_0, M_1 and M_2 generations of *Helianthus annuus* L. variety Ekiz 1

Generation	Treatment	Total no. of cells scored	Total no. of dividing cells	Mitotic index ± SE	Total no. of abnormal cells	Total no. of abnormal cells (% ± SE)
	Control	2565	437	17.60 ± 1.11	5	1.35 ± 0.79
M_0	10 kR	3346	505	15.17 ± 0.31*	34	6.74 ± 0.99**
	20 kR	3349	476	14.35 ± 0.57**	39	8.48 ± 1.68**
	30 kR	3932	552	14.10 ± 0.38**	58	10.83 ± 0.55**
	40 kR	3699	501	13.55 ± 0.50**	75	15.13 ± 1.23**
	50 kR	4220	552	13.09 ± 0.48**	106	18.65 ± 2.39**
	Control	3889	696	17.86 ± 0.44	3	0.72 ± 0.39
M_1	10 kR	3799	663	17.39 ± 0.51	25	3.88 ± 0.72**
	20 kR	3464	505	14.57 ± 0.36**	28	5.35 ± 0.85**
	30 kR	3097	429	13.78 ± 0.58**	31	7.11 ± 1.40**
	40 kR	3236	422	13.09 ± 0.54**	41	9.47 ± 2.07**
	50 kR	3546	460	13.04 ± 0.46**	56	11.80 ± 1.23**
	Control	6705	1143	17.38 ± 1.17	4	0.52 ± 0.29
M_2	10 kR	6241	1112	17.04 ± 0.75	27	4.22 ± 1.99**
	20 kR	4523	714	15.88 ± 0.37*	29	3.49 ± 0.66**
	30 kR	2770	454	15.73 ± 0.81*	33	7.88 ± 1.61**
	40 kR	3746	567	14.92 ± 0.64**	35	6.05 ± 1.91**
	50 kR	3059	471	14.31 ± 0.63**	42	8.79 ± 1.24**

*Significant at 5% level (t-test)
** Significant at 1% level (t-test)

DISCUSSION

The decrease in the mitotic index of some other plants depending on the radiation dose has been noted previously by other authors. Sapparow *et al.* (1952) claim that mitotic inhibition may be related to physiological disturbances caused due to chromosome break in a nucleus. Tarar and Dynansagar (1983) assert that the fall in mitotic index seems to have been caused by a physiological change brought about by the gamma rays in the viscosity of cytoplasm, which might have inhibited the synthesis of hormones, enzymes and nucleic acid.

The decrease in the mitotic index at all doses according to the control noted in this study might to brought about due to partial interference or postpone of RNA, DNA and protein synthesis and spindle by gamma radiation. The reason of the occurance of C-metaphase, which has been observed in all of the generations in this study might be either partial interference or postpone of chromosome's centromers or spindles and their functions.

Table 2: Percentage of anomalies occuring during mitosis in control and in gamma-irradiated samples of *Helianthus annuus* L. variety Ekiz 1

Generation	Treatment	Total number of dividing cells	METAPHASE			ANAPHASE		
			C-met-aphase (%)	Stickness (%)	Laggard (%)	Bridge (%)	Laggard (%)	Birdges with fragments (%)
M_0	Control	437	0.46	-	-	0.69	-	-
	10 kR	505	2.97	-	0.40	2.77	0.40	0.20
	20 kR	476	1.47	0.21	0.21	6.09	0.20	-
	30 kR	552	1.45	-	0.36	8.51	0.18	-
	40 kR	501	4.19	0.20	0.20	7.78	1.40	1.20
	50 kR	552	3.26	0.18	1.81	11.78	1.45	0.72
	Control	696	0.29	-	-	0.14	-	-
M_1	10 kR	663	1.51	-	0.15	1.66	0.45	-
	20 kR	505	2.97	0.20	-	1.98	0.40	-
	30 kR	429	4.43	0.23	-	2.56	-	-
	40 kR	422	5.69	0.24	-	3.08	0.71	-
	50 kR	460	4.57	1.74	0.43	5.22	0.22	-
	Control	1143	0.17	-	-	0.17	-	-
M_2	10 kR	1112	0.45	-	0.54	1.26	0.09	0.09
	20 kR	714	1.12	0.14	0.84	1.12	0.42	0.42
	30 kR	454	1.54	-	0.44	4.19	0.88	0.22
	40 kR	567	2.47	-	0.35	2.29	0.88	0.18
	50 kR	471	2.55	1.49	0.64	3.82	0.21	0.2

The chromosomal stickness noted by Darlington and La Cour (1940) as a result of the depolimerization of nucleic acids after radiation has rarely been observed in the present study. This abnormality type has been noticed as sticky chromosomes in metaphase and sticky bridges in anaphase. Stickness can be supposed as physiological events due to changings in the chromosomes at nucleo-protein level or changings of proteins due to abnormal sequence of chromosome fibres.

Although observations are possible at mitotic metaphase in the most plant species, anaphases are better suited for quantitatively recording chromosome mutations (Anonymus, 1977).

In this study, the dominant abnormality has been the bridges with or without fragments at anaphase stage most of which have been double bridges. Caldecott and Smith (1952) report that after ionizing radiation, all or most of the bridges occur as double bridges. Evans (1962) attributed that structural alternations of chromosomes in the irradiated materials are concequences of breakage and rejoining. Sax (1941) claimed that the breaks induced in chromosomes after irradiation might be due to a change in the molecular constituents of the chromosomes. Swanson (1965) says that fragments associated with bridges appeared due to paracentric inversion. The cause of bridge formation is in the fusing of two centromere-bearing choromo-

some fragments. After the effective chromosome splitting into two chromatids tend to go to opposite poles and a bridge is formed which breaks sooner or later (Anonymous, 1977).

Caldecott *et al.* (1954) and Gaul (1963) reported that the frequency of these bridges is directly proportional to the dose of both X-rays and neutrons. Similarly, in this study the number of the bridges has increased parallel to the dose increase in all of the generations.

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LOS RAYOS DE GAMMA CAUSA ANORMALIDADES MITOTICAS EN *Helianthus annuus* L. VARIEDAD DE EKIZ 1

RESUMEN

En este estudio, el efecto de los rayos gammas en Ekiz l, varieda que pertenecia al *Helianthus annuus* L. en las generaciones de M_0 (germenes irradiados primeros), M_1 y M_2 ha sido investigado. Germenes fueron irradiados con los rayos gammas al 10, 20, 30, 40 y 50 Kr dosises. El porcentaje de los

anormalidades totales de generaciones de M_0, M_1 y M_2 ha sido aumentado paralelo al aumentode la dosis de la radiacion. Estos anormalidades ha sido observadocomo c-metaphase, viscosidad de la cromosoma, los rezagados y fuentes con fragmentos y sin fragmentos. El indice mitotico en las generaciones de M_0, M_1 y M_2 ha sido disminuido paralelo al aumento, de la dosis. Cuando la generacion se compara entre una a otra ambos la cantitad de disminucion en el indice mitotico y el porcentaje de anormalidades mitoticos se observan sobre todo en la generacion de M_0.

LES ANORMALITÉS MITOTIQUES CHARGÉÉS DES RAYONS GAMMA

RESUME

Dans cette étude, les influences mitotiques des rayons gamma ont eté examinéés dans les generations M_0 (les premiers germes radiants), M_1 et M_2 de la variété Ekiz 1. appatenant â *Helianthus annuus* L. (2n=34). Les germes (M_0) ont été rayonnés avec les rayons gamma d'une dose de 10, 20, 30, 40 et 50 kR. Dans les générations M_0, M_1 et M_2, le pourcentage d'anormalité total paralléle a la dose de radiation a augmenté (es anormalités ont été observées en qualité de pont avec fragments et sans fragments, de laggards, de chromosomes adhésives et de c-metaphase). Dans lés générations M_0, M_1, et M_2 1'index mitotic a diminué paralléle â 1'augmetation de la dose. Lorsque les générations se rencontraient, non seulement il ya eu une quantité de diminution dans 1'index mitotic, mous encore le pourcentage de 1'anormalité mitotique a été observé le plus dans M_0.