

## MEIOTIC STUDIES IN THE M<sub>2</sub> GENERATION OF *Helianthus annuus* L. VARIETY EKIZ 1 AFTER GAMMA IRRADIATION

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### SUMMARY

The effect of gamma rays on meiosis in the M<sub>2</sub> generation has been studied in sunflower (*Helianthus annuus* L.) variety Ekiz 1. Seeds were irradiated with gamma rays at 10, 20, 30, 40 and 50 kR doses. Radiation induced meiotic abnormalities. The chromosomal aberrations included univalents, multivalents and stickiness at diakinesis, laggards and stickiness at metaphase I, bridges and laggards at anaphase and telophase I-II, micronuclei at telophase II. The percentage frequencies of anomalies increased generally with the increasing dose of radiation.

**Key words:** *Helianthus annuus* L., meiotic abnormalities, gamma rays

### INTRODUCTION

Cytogenetic investigations are essential for obtaining information concerning the roles and effects of mutagens on various genotypes. Gamma irradiation is one of the most important physical mutagens applied to induce cytogenetic changes and mutation in plant. Eventhough there are several reports that consider *Helianthus annuus* L. (2n=34) as a precious plant in terms of fat, the studies on meiotic aberrations caused by chemical and physical mutagens were not frequent (Chand *et al.*, 1991; Arslan *et al.*, 1994).

In the present investigation, meiotic abnormalities induced by gamma radiation have been studied in the M<sub>2</sub> generation of *Helianthus annuus* L. variety Ekiz 1. The variety has high yields of fat in seeds and resistance to *Orobanche cumana* in the conditions of Turkey.

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Table 1: Induced meiotic chromosomal abnormalities in the M<sub>2</sub> generation of *Helianthus annuus* L. variety Ekiz 1 at different dose treatments

Dose	Diakinesis				Metaphase I			Anaphase-telophase I			Anaphase-telophase II			
	No. of cells observed	Univalents (%)	Multivalents (%)	Stickiness (%)	No. of cells observed (%)	Laggards (%)	Stickiness and clumping	No. of cells observed	Laggard (%)	Bridges (%)	No. of cells observed	Laggards (%)	Bridges (%)	Micronuclei (%)
Control	96	-	-	-	680	1.17	-	527	1.13	0.37	306	0.32	0.32	
10 kR	33	3.03	3.03	-	417	2.87	3.11	677	1.32	1.77	355	0.56	1.12	1.69
20 kR	103	1.94	3.88	4.85	591	1.52	1.86	1012	2.96	1.97	188	1.59	2.66	0.53
30 kR	132	3.03	8.33	2.27	442	2.48	2.26	436	5.04	3.66	176	1.13		
40 kR	66	5.57	4.54	-	262	2.67	7.63	603	3.64	1.32	294	1.36	0.68	
50 kR	59	5.08	13.55	8.47	499	3.60	4.00	671	2.53	4.32	187	1.60	3.20	1.60

## MATERIALS AND METHODS

Seeds of *Helianthus annuus* L. (Ekiz 1) were irradiated with gamma rays from a  $^{60}\text{Co}$  source. The doses employed were 10, 20, 30, 40 and 50 kR. The treated seeds were sown along with the control in the field in three replicates according to complete random block design. Each  $M_1$  plant was harvested separately and  $M_2$  plants were raised from  $M_1$  seeds. Meiotic studies were conducted on ten randomly selected plants from each treatment.

For meiotic studies, parts of young capitulum (before opening) were fixed in freshly prepared Carnoy's fluid (ethanol:acetic acid, 3:1) for 24 h between the hours 8.00 AM and 10.00 AM of each day and then transferred to 70% ethanol and stored in a refrigerator till use. Pollen mother cells were examined by making smears in 1% acetocarmine. Photographs were taken from permanent preparations using a Olympus BHS microscope with a photomicrographic attachment.

## RESULTS

Different types and frequencies of meiotic chromosomal aberrations were observed during microsporogenesis in the  $M_2$  generation of *Helianthus annuus* L. at various dose treatments (Table 1). The total meiotic irregularities at various doses of radiation and control are presented in Table 2.

Table 2: The total meiotic abnormalities in the  $M_2$  generation of *Helianthus annuus* L. variety Ekiz 1 at different dose treatments

Dose	Total number of PMCs	No. of PMCs with abnormalities	% of PMCs with aberrations
Control	1609	18	1.12
10 kR	1482	60	4.04
20 kR	1894	90	4.75
30 kR	1186	79	6.66
40 kR	1225	71	5.79
50 kR	1416	112	7.91

At diakinesis 17 bivalent were regularly observed in the control plants (Figure 1a) whereas the treated plants showed both bivalents and associations of three, four and five chromosomes (rings, rods and chains) (Figure 1b).

Majority of the abnormal cells were characterized by lagging chromosomes at metaphase I and ana-telophase I (Figure 1d) and ana-telophase II (Figure 1g) bridges at ana-telophase I and II (Figures 1e, f, g) stickiness or clumping of chromosomes at diakinesis and metaphase I (Figure 1c) micronuclei at telophase II (Figure 1h).

In control samples, cells with lagging chromosomes and sticky bridges were observed at low frequencies. Meiotic abnormalities increased generally with the increasing dose of radiation in the  $M_2$  generation.

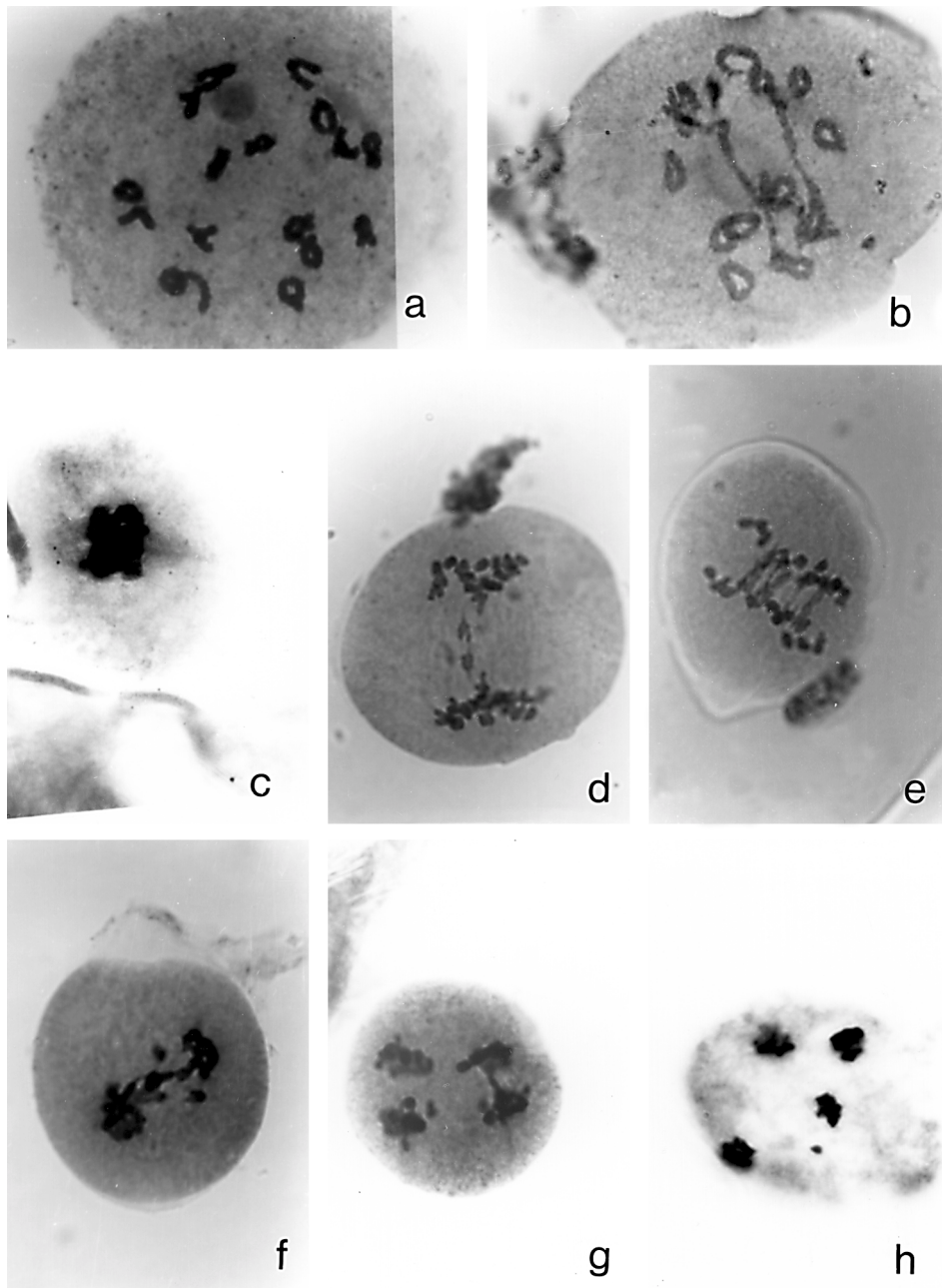


Figure 1: Behaviour of meiotic chromosomes (a) diakinesis showing 17 bivalents in control plants; (b) multivalents at diakinesis; (c) stickiness of metaphase I; (d) laggard at anaphase I; (e) bridges at anaphase I; (f, g) laggard with bridges at telophase I and telophase II; (h) micronuclei at telophase II (x 1100)

## DISCUSSION

The meiotic abnormalities recorded in the present study have also been reported in plants raised from irradiated or chemically treated plants (Evans, 1962; Das and Roy, 1989; Chand *et al.*, 1991; Savaşkan *et al.*, 1991, Arslan *et al.*, 1994).

The frequencies of chromosomal abnormalities were considerably reduced in the  $M_2$  generation as compared with the  $M_1$  generation (Arslan *et al.*, 1994) at all dose treatments. For instance, although percentage of meiotic abnormalities in the  $M_1$  generation was 6.50% at 10 kR and 22.84% of 50 kR, in the  $M_2$  generation it was 4.04% at 10 kR and 7.91% at 50 kR.

Das and Roy (1989) observed that the percentage of frequencies of abnormalities was more numerous in  $M_1$  than in  $M_2$  due to genetic recovery or elimination of the defectives in their *Solanum* radiocytogenetic studies. Savaşkan *et al.* (1991) suggested that the lowered values in  $M_3$  are due to the elimination of the sterile and self-sterile plants in  $M_2$  of soybean.

Meiotic abnormalities observed in control of the  $M_2$  generation (1.12%) is the sign of not having genetic stability in this plant, which is allogamous. This situation can be attributed to the fact that hybrid seeds were sown for two years.

In  $M_2$ , the univalent and multivalent formation was less as compared with  $M_1$  at the same dose (e.g., multivalent 39.22% at 50 kR in  $M_1$  and 13.55% at 50 kR in  $M_2$ ). The occurrence of trivalents, chain and ring multivalents demonstrates that mutagenesis resulted in structural alterations leading to the rearrangement of chromosomes.

The laggards observed in the present investigation as reported by a large number of workers may be due to delayed terminalization stickiness of chromosome ends or because of the failure of chromosomal movement. Evans (1962) claimed that laggards appeared due to abnormality in the spindle or due to acentric fragments.

Radiation inducing chromosome stickiness was reported to be the result of partial disassociation of the nucleoprotein and alteration in their pattern of organization (Evans, 1962).

The bridges occurring in this study might be attributed to the breaking and reunion of the chromosomes or to the stickiness of the chromosomes at metaphase.

Jackson (1988) suggested that some of the bridges may have been caused by a crossover between a paracentric inversion heterozygote loop and the centromere and another in the loop of the same bivalent. In addition, he asserted that chromatin bridges without fragments may have resulted from linear disjunction of multivalents.

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## REFERENCES

- Arslan, O., Ş. Bal, N. Yenice, S. Mirici and S. Pekol, 1994. Meiotic studies in  $M_1$  generation of sunflower (*Helianthus annuus* L.) treated with various doses of gamma rays. XII National Biology Congress, 6-8 July, Edirne, Volume 5, pp. 140-146.
- Chand, H., R.K. Grover and P.K. Sareen, 1991. Carbendazim induced chromosomal aberrations in somatic and reproductive cells of pearl millet and sunflower. *Cytologia*, 56, 559-566.
- Das, S.K., and S.K. Roy, 1989. Radiocytogenetical studies on *Solanum* L. meiotic abnormalities. *Cytologia*, 54, 477-481.
- Evans, H.J., 1962. Chromosomal aberrations induced by ionizing radiations. *Int. Rev. Cytol.*, 13, 221-321.
- Jackson, R.C., 1988. A quantitative cytogenetic analysis of an intersectional hybrid in *Helianthus* (*Compositae*). *Amer. J. Bot.*, 75 (5), 609-614.
- Savapkan, C., A.S. Atilla, and Z. Sađel, 1991. Chromosomal aberration induced by gamma irradiation in *Glycine max* L. *Turkish Journal of Nuclear Sciences*, Vol. 18, No. 2, 29-34.

### **LOS ESTUDIOS EN LA GENERACION DE *Helianthus annuus* L. LA VARIEDAD EKIZ 1 DESPUES DE LA IRRADIACION DE LA GAMMA**

## RESUMEN

El efecto de los rayos gammas en meiosis en la generación de  $M_2$  se han estudiado en la variedad Ekiz 1 del girasol. Los germenos fueron irradiados con los rayos gammas al 10, 20, 30, 40 y 50 kR dosis. La radiación causa a algunas anomalías meióticas. Las desviaciones de las cromosomas incluyeron univalent, multivalent y viscosidad en diakinesis, los rezagados y viscosidad en metaphase I, los fuentes y rezagados en anaphase y telophase I-II, micronuclei en el telophase II. El porcentaje de frecuencia de los anomalías se aumenta generalmente con la dosis de aumento de la radiación.

### **APRÉS LE RAYONNEMENT DES GAMMA DANS LA GÉNÉRATION $M_2$ DE LA VARIÉTÉ EKIZ 1 (*Helianthus annuus* L.)**

## RÉSUMÉ

Dans la generation  $M_2$ , l'influence des rayons gamma sur la mayose de la variété Ekiz 1 de *Helianthus annuus* L. (tournesol) a été recherché. Les germes ont été radiationnés d'une dose de 10, 20, 30, 40 et 50 kR. La radiation a été cause des anomalies méiotiques. Les aberrations chromosomiques, l'univalent dans la diakinésie, les multivalent et l'adhésivité, des laggards et des ponts dans la télophase et l'anaphase I et II; et dans la télophase II les micronucléi ont été observés. La densité du pourcentage des anomalies en général a augmenté avec l'augmentation de la dose de radiation.