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Research Note

The Effects of Gamma Irradiation on the Pollen Size of *Gossypium hirsutum* L.

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Abstract: *Gossypium hirsutum* L. (cotton) is cultivated widely in the south of Turkey. In this study, seeds of the cotton cultivar Çukurova 1518 were irradiated with gamma at 50, 100, 200, 300 and 400 Gray doses in a Co^{60} source. The size of morphologically normal pollen grains was measured in generation M_1 and found to be 120.35 µm in the control group, while this value was different in the irradiated groups. However, there was no significant difference in the size of pollen grains of normal morphology and shape between the irradiated and control pollens.

Key Words: *Gossypium hirsutum* L., gamma irradiation, pollen size, pollen morphology

Gama İle Işınlamanın *Gossypium Hirsutum* L. Polen Büyüklüğü Üzerine Etkileri

Özet: *Gossypium hirsutum* L. (pamuk) Türkiye'nin özellikle güney bölgesinde yaygın olarak yetiştirilmektedir. Bu çalışmada Çukurova 1518 çeşidine ait pamuk tohumları bir Co^{60} kaynağında 50, 100, 200, 300 ve 400 Gy dozlarında gama ile ışınlandı. Normal polen büyüklüğü M_1 generasyonunda ölçüldü ve ışınlanmış gruplarda farklı değerlerde olurken kontrol grubunda 120.35 µm olarak bulundu. Ancak ışınlanmış gruplara ait polen büyüklüğü ve şekli ile kontrol grubuna ait polenler arasındaki fark istatistiksel olarak önemsiz bulundu.

Anahtar Sözcükler: *Gossypium hirsutum* L., gama ışınlama, polen ölçümü, polen morfolojisi

Introduction

Gamma radiation is one of the main physical mutagen for mutation studies in plants or any other techniques related with irradiation. Mutagens have been effective in decreasing the mitotic index (Savaşkan & Toker, 1991) or increasing micronuclei number and pollen abnormalities (Mehetre & Thombre, 1981; Giles & Prahash, 1987; Savaşkan & Atila, 1991). The control of plant reproduction has a high priority in the use of genetic engineering in agriculture. On the other hand, the practical applications of pollen research are essential for understanding the biological mechanisms of the determination of fertility (Bedinger, 1992). Penetration of different types of irradiation (uv, gamma, X-rays) varies in species, depending on the size and shape of the pollen grain, and the thickness of the pollen wall (Giles & Prakash, 1987).

Cotton (*Gossypium hirsutum* L.) is one of the most important crops of Turkey and is cultivated mainly in the southern part of Anatolia. Some of the important qualitative characters of cotton are related to fibre features such as length and strength (Şenel, 1980). They can be changed using mutation techniques for higher quality (Özbek et al., 1991). Heteromorphic pollen grains or abnormal structures after unsuccessful division, such as in Figure 1, do not have an effect on reproduction because of elimination while pollen is responsible for fertilization in further generations. The percentage of abnormal pollen grains of cotton in generations M_1 and M_2 were observed and a higher amount of abnormalities

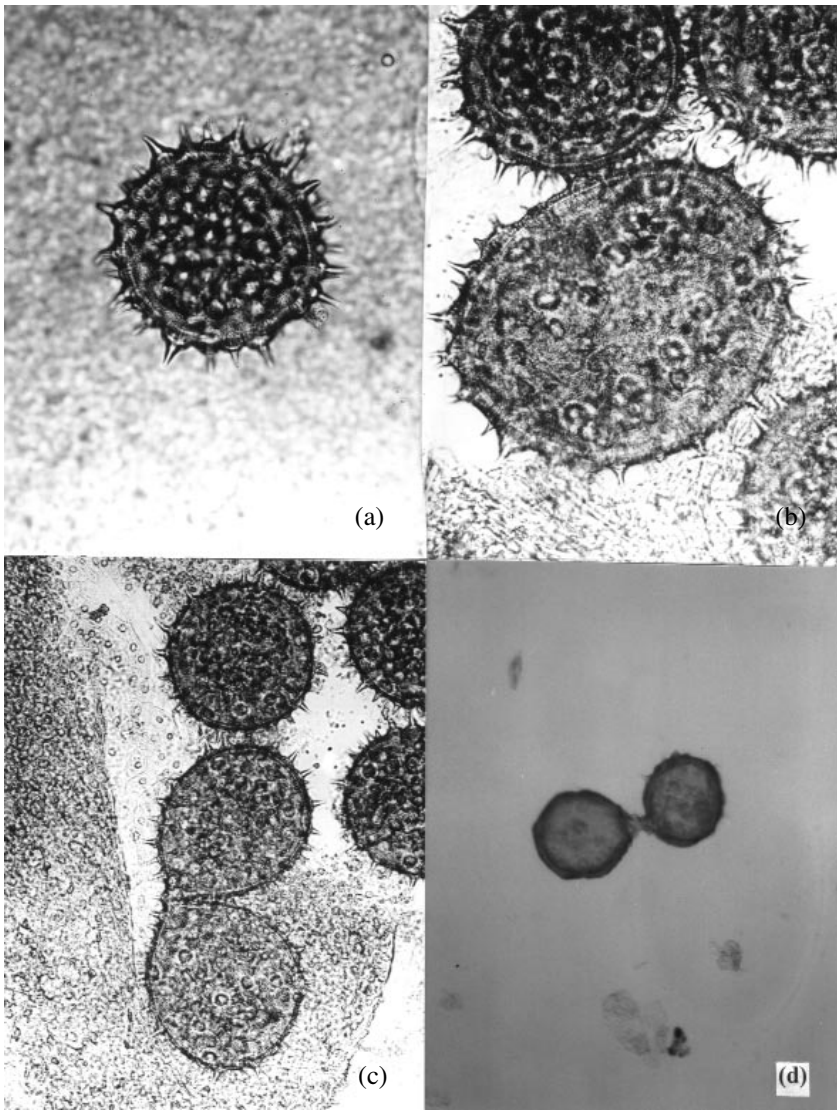

Figure 1. (a) A normal pollen grain of *Gossypium hirsutum* L. cv. Çukurova 1518 (cotton) taken from the control group, (b) a heteromorphic pollen grain affected by irradiation with 300 Gy gamma doses, above, (x 200). Also two different views from cotton pollen grains which unsuccessfully divided in the irradiated groups with 300 Gy doses (x 100) (c), 400 Gy doses (x 35) (d), below.

was found at higher doses (Savaskan & Atila, 1991). The objective of this study was to determine the size of pollen grains of normal shape and morphology after irradiation with various doses of gamma radiation.

Materials and Methods

Seeds of *G. hirsutum* L. cv. Çukurova 1518 (2n = 4x = 52) were treated with 0, 50, 100, 200, 300 and 400 Gy doses of gamma rays from a Co^{60} source and sown in the Field Crops Research Institute fields in Adana. Bud specimens were collected in M_1 plants in the second week of June. Buds were fixed (1 propionic acid:2 absolute alcohol) for 24 h, and then they were rinsed and transferred to 70% alcohol and stored in a refrigerator (+4 ºC) until use. Anthers of buds were smeared in McCallum's propionocarmine solution (1%) (Mehetre & Thombre, 1981). Pollen axis (P) and total breadth (E) were measured with an ocular micrometer mounted on a light microscope (Zeiss MC 63) in both the control and irradiated groups. Measurements of pollen grains were obtained according to the formula below (Faegri & Iversen, 1989).

$$M = m + a \times 1/n \, \Sigma xy$$

In this formula, m refers the unit as it has the greatest number of pollen grains, n refers to the total amount of pollen measured, Σxy is the correction factor (Table 2)

and a is equivalent to 1 (constant). The ocular unit value (6.15 µm) was multiplied by the M value in order to determine the diameters of pollen grains. A chi square (X^2) test was performed for the pollen grain size measured in the groups (Table 3).

Results and Discussion

The sizes of pollen grains were mostly from 11 to 27, from 11 to 25 and also from 15 to 27 ocular units in the groups irradiated with 300, 200 and 400 Gy doses respectively (Table 1). In the control group, pollen grains were accumulated particularly in 19 and 20 ocular units (Table 1). Mehetre & Thombre (1981) observed that unequal separation of chromosomes and chromatids at anaphase I and II led to the formation of abnormal tetrads and pollen grains with high size variations leading to high pollen sterility. The normal pollen size of cotton in the control group was 120.35 µm (Figure 1), (Table 3). Mehetre & Thombre (1981) found that diploid pollen size was 117.3 µm in the control group of an Indian cotton variety H.G.180. In addition, variety and genotype actors may cause this difference. Also, the external characters of pollen can be influenced by temperature (climatic conditions), mineral nutritions or soil water conditions (Stanley & Linskens, 1974).

The pollen size in the control group was higher than that in the groups irradiated with 200, 300 and 400 Gy doses and lower than that in the groups irradiated with 50 and 100 Gy (Table 3). However, there was no significant difference in the values between the control and irradiated groups (Table 3). According to these results, doses higher than 400 Gy can increase the size changes of pollen grains and cause more sterility or mortality in plants. In this study, these chosen doses were found to be suitable for mutation works in cotton to produce mutant lines for high yield and quality (Özbek et al., 1991). With the same doses of gamma radiation, they produced 4320 single plants in M_2 and 160 mutant lines in generation M_3 for high yield and long fibre with the

Table 1. Distribution of pollen size of *Gossypium hirsutum* L. (cotton) in each of control and irradiated groups (Savaskan & Atila, 1991).

Irradiated Groups (Gy)	Units (per ocular unit = 6.15 µm)																	Total pollen number
	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	
0	-	-	-	-	-	-	1	-	53	37	8	1	-	-	-	-	-	100
50	-	-	-	-	-	-	-	8	41	37	8	4	1	-	1	-	-	100
100	-	-	-	-	1	-	3	17	35	26	12	1	5	-	-	-	-	100
200	1	-	-	-	4	7	14	19	21	21	3	4	5	-	1	-	-	100
300	2	-	1	4	15	18	29	13	13	3	1	1	1	-	1	-	1	103
400	-	-	-	-	3	20	20	20	14	12	7	3	2	1	-	-	1	103

Table 2. Correction factors according to the distribution of pollen size of cotton in the control and irradiated groups.

Irradiated Groups (Gy)	Total pollen grains evaluated	Correction factors	
		Σxy (*P)	Σxy (*E)
0	100	55	57
50	100	72	72
100	100	37	37
200	100	46	45
300	103	4	2
400	103	64	62

* P = pollen axis, E = total breadth

Table 3. The pollen size of *Gossypium hirsutum* L. (cotton) in the control and irradiated groups.

Irradiated Groups (Gray)	Dimensions of pollen grains			Shape classes
	*$P_{M \times 6.15}$ µm	*$E_{M \times 6.15}$ µm	P/E	
0 (Control)	120.35	120.35	1.000	spheroid (1.14-0.88)
50	121.27	121.27	1.000	"
100	125.27	125.27	1.000	"
200	119.67	119.61	1.001	"
300	104.79	104.67	1.001	"
400	108.36	108.30	1.001	"

*P = pollen axis, E = total breadth, P (0.05) > 3.547n.s., sd = 5

parent variety Çukurova 1518 (Özbek et al., 1991). In total, in M_1 16539 pollen grains and in generation M_2 7651 pollen grains were observed for the percentage of abnormalities in the control and irradiated groups by Savaşkan & Atila (1991). They found that abnormalities and heteromorphic pollen grains increased in generations M_1 and M_2 from 1.6% in 50 Gy to 10.39% in 400 Gy doses and from 0.61% in 50 Gy to 7.10% in 400 Gy doses, respectively (Savaşkan & Atila, 1991). This study showed that there were changes in the size of remaining pollen grains in the normal shape or morphology in generation M_1. The degrees of these changes are going to decrease in further generations by conscious selection of plants or naturally.

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