THE EFFECT OF GAMMA RAYS ON POLLEN VIABILITY, GERMINATION AND POLLEN TUBE LENGTH IN SAKI APPLE CULTIVAR

GAMA IŞINLARININ SAKI ELMA ÇEŞİDİNE AİT POLENLERİN CANLILIK, ÇİMLENME VE POLEN TÜPÜ UZUNLUKLARINA ETKİSİ

Demet YİĞİT[1*], Muharrem GÜLERYÜZ[2], Ercan BALCI[3]

[1] Department of Science Education, Education Faculty, Erzincan University, Erzincan, 24030, Turkey*
[2]Department of Horticulture, Agriculture Faculty, Atatürk University, Erzurum, 25240, Turkey
[3] Department of Radiation Oncology, Medicine Faculty, Atatürk University, Erzurum, 25240, Turkey

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ABSTRACT

In this study, the effects of different doses of gamma rays (Co^{60}) on Sakı apple (*Malus domestica*) pollen viability, germination and pollen tube length were investigated, *in vitro*. With this aim, five different ionizing radiation doses (5, 10, 20, 25 and 50 krad) were treated. While the irradiation with low gamma doses (5 and 10 krad) affected the pollen germination and pollen tube length, affirmatively; irradiation at a range of 20-50 krad doses affected the same parameters negatively. There was a highly significant inverse relationship between gamma dose and germination percentage; the LD50 was about more than 50 krad.

Keywords: Gamma rays, pollen viability, pollen germination, pollen tube length.

ÖZET

Bu çalışmada, değişik dozlarda uygulanan gama ışınlarının (Co^{60}) Sakı elma (*Malus domestica*) çeşidine ait polenlerin *in vitro* şartlarda canlılığına, çimlenmesine ve pollen tüpü uzunluğuna etkisi araştırılmıştır. Radyasyon dozları olarak 5, 10, 20, 25 ve 50 krad dozları uygulanmıştır. 5 ve 10 krad'lık düşük gama dozları pollen çimlenmesini ve pollen tüpü uzunluğunu olumlu yönde etkilerken, 20-50 krad'lık dozlar aynı faktörler

*Corresponding author: demyigit@hotmail.com

üzerine negatif etki göstermiştir. Uygulanan gama dozları ile çiçek tozu çimlenme yüzdesi arasında negatif bir korelasyon tespit edilmiş ve LD50 değeri 50krad'dan yüksek bulunmuştur.

Anahtar Kelimeler: Gama ışınları, polen canlılığı, polen çimlenmesi, polen tüpü uzunluğu.

1. INTRODUCTION

Important indices of male gametophyte functional ability are pollen germination capacity and the rate of pollen tube growth (Mratinic, 1996). Aside from genotype, these characteristics are strongly influenced by some ecological factors such as temperatures, relative humidity, solar radiation, etc. (Zebrowska, 1997). A number of studies have been carried out to clarify the influence of the ionizing radiation of pollen in various species (Aly, 1995; Savaşkan, 2002; Sanamian, 2003; Wang *et al*., 2007; Wi *et al*., 2007).

In practice, nowadays, most often use is made of sparsely ionizing radiation, to which category X-rays and gamma rays belong. Gamma rays and X-rays are electromagnetic radiations and have an energy level that is high enough, to ionize atoms in molecules with which they interact. Gamma rays have shorter wavelengths, but otherwise are identical with X-rays with respect to their physical properties.

Most studies of pollen radiosensitivity have been conducted deal with the effect of irradiation on pollen germination and pollen tube growth (Abak *et al*., 1997; Todorova *et al*., 2004; Kumar and Rai, 2006; Gonai *et al*., 2006).

It is known that ionizing radiations inhibit pollen germination only at very high doses Irradiation experiments of binucleate pollen species showed a high radioresistance for pollen germination (Pfahler, 1971). According to Visser and Oost (1981), the dose causing a 50% reduction of pollen germination (LD50) averaged 220 krad for *Malus domestica* and *Pyrus communis*, both belonging to the Rosaceae family.

Relatively little attention has been paid to the stimulary effect of low doses of radiation. Radiation induced stimulation of different biological objects and parameters such as plant growth and yield

(Banerji *et al*., 1996; Sümer, 1996; Gülsen *et al*., 2007), pollen viability, pollen germination, pollen tube elongation (Sperenza *et al*.,1982; Tuyl *et al*.,1982) and biochemical products (Calzoni and Sperenza 1982; Georgieva and Atanassov, 1986) was reported by some early workers.

While various studies that shows to effect of irradiation on pollen germination and pollen tube growth, ours is the first study demonstrating these effects on *Malus domestica* cv. Sakı. So this study was designed to examine the effect of gamma ^{60}Co irradiation on apple pollen viability and germination rates and pollen tube growth, *in vitro*.

2. MATERIALS AND METHODS

Collection and storage of pollen

Pollen of *Malus domestica* cv. Sakı was obtained from 15 years old trees in Erzincan, Turkey. Flowers were cut from the trees in the balloon stage, and placed on desk at room temperatures. Flowers with all anthers dehisced (about 3-4 days) after anthesis and at the begining of petal fall) were carefully removed using forceps, so that pollen was not lost. The stamens were vibrated with forceps so the pollen fell on to the sheet and was easily collected in eppendorf tubes and stored at –20^{o}C ± 2 until for the irradiation treatments (Yiğit, 2000).

Irradiation procedure

Gamma irradiation of apple pollen was performed with a ^{60}Co source at Oncology Department of Medicine Faculty in Atatürk University. The dry pollen of Sakı apple was treated in eppendorf tubes with 5, 10, 20, 25 and 50 krad doses.

Pollen viabilty, pollen germination and pollen tube growth

In vitro pollen germination trials were carried out on a medium with 1% agar, 10 % sucrose and 0.1% boric acid. After an incubation period of 12 h at 22^{o}C ± 2, germination was determined (Marcucci *et al*., 1984). Pollen grains were cultured in duplicate and the experiment was repeated 8 times. For each petri dish, about 300 pollen grains and 150 pollen tubes were recorded. Pollen grain germination was considered to occur *in vitro* when a pollen tube had

grown at a length at least twice the diameter of pollen grain. Pollen tube length measurements were made at 100X magnification (Ozawa *et al*., 1993). Pollen viabilty was measured by staining the pollen with TTC (2, 3, 5 Triphenol tetrazolium chloride) and recorded as the number of viable and nonviable grains and converted to a percentage for analysis (Yiğit, 2000). A micrograph of viable apple pollen is shown in Fig 1.

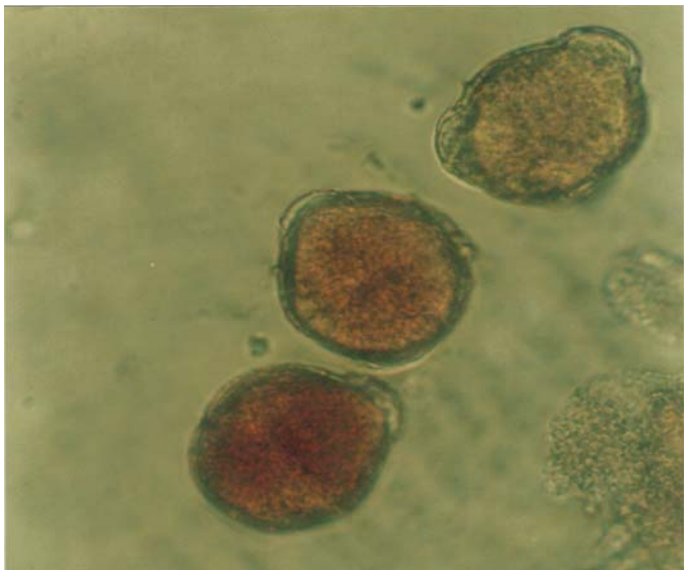

Figure 1. A micrograph of Sakı apple pollen after staining TTC, (X400).

Statistical analyses

Statistical calculations were done by using SPSS 12.0 programme. To be able to determine the statistical significances of pollen viability, pollen germination and pollen tube length results, one-way variance analyses were applied, and then multiple comparisons were carried out by LSD test.

3. RESULTS

The effects of gamma rays treated at different doses on apple pollen viabilty and germination rates (%) and pollen tube length (µm) are shown in Table 1 and Fig.2.

Table 1. The effects of gamma rays treated different doses on Sakı apple pollen viability and pollen germination rates (%) and pollen tube length (µm).

Gamma Doses (Krad)	Pollen Viability Rate (%)	Pollen Germination Rate (%)	Pollen Tube Length (µm)
0	68.2b *	70.4ab	826.3a
5	70.7ab	72.3a	830.9a
10	72.3a	74.6a	828.4a
20	72.8a	68.2b	790.2b
25	71.3a	58.1c	636.4c
50	67.2b	40.4d	372.8d

*: Within columns, means followed by the different letters are significantly different ($p < 0.05$) by LSD test.

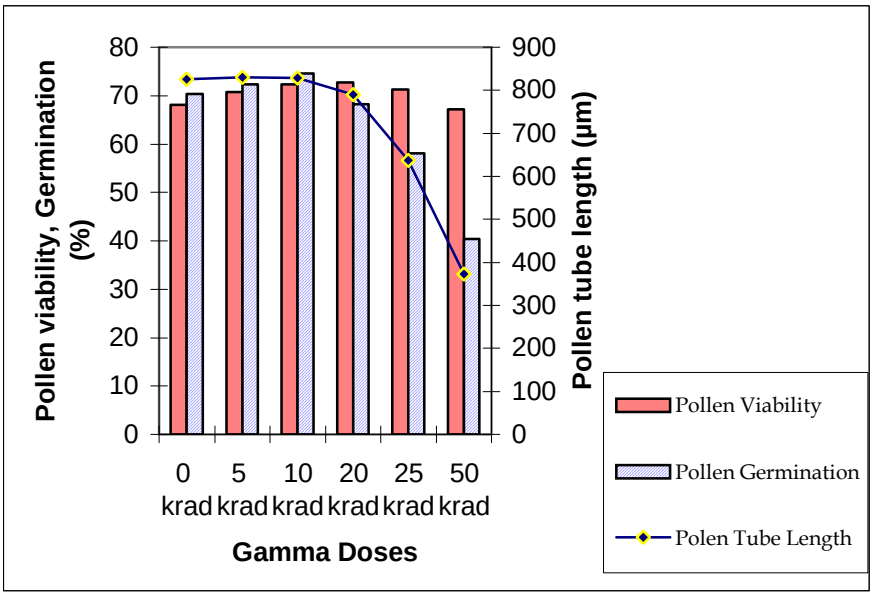

Figure 2. The influence of gamma ray treatment at different doses on pollen viability and germination (%) and pollen tube length (µm) of Sakı apple.

The irradiation of pollen with 5 and 10 krad gamma rays stimulated pollen germination but the pollen germination values were not significantly different than control (70.4%) (P >0.05). After irradiation with doses 25 and 50 krad, the rate of pollen germination markedly decreased. The LD50, taking germination at 70.4% (control), was about more than 50 krad (Table 1).

The doses (5 and 10 krad) stimulating the pollen germination, also stimulated the pollen tube length. But pollen tube length was only slightly affected by these irradiation doses, and are not significantly different from control (826.3 µm, P >0.05). After irradiation with a dose higher than 20 krad, the pollen tube length was seriously decreased. There was a significant negative correlation (r =–0.953) between pollen tube length and gamma doses (Table 2).

Table 2. Correlation coefficients amoung gamma dose, pollen viability, pollen germination and pollen tube length.

	G.Dose	**Viability**	**Germination**	**Pollen tube Length**
G. Dose	_	– .319	–.881**	–.893**
Viability		_	.588	.559
Germination			_	.983**
Pollen tube Length				_

**: It is significant at 0. 01 level

We used correlation matrices to assess whether the survival responses (pollen germination, pollen tube length and pollen viability) of Sakı apple pollen differed with gamma-ray treatment. Both pollen germination and pollen tube length were significantly negative correlated with gamma doses (r = –0.939 and r = –0.953, respectively).

The pollen viabilty, as measured by staining increased with increasing the irradiaton doses. Although a slight decrease shown in pollen viability (67.2%) at 50 krad radiation dose, no statistically significant difference could be determined between control and this dose. Following the irradiation in the range of 5-25 krad, the pollen viability was significantly affected, somewhat higher doses stimulated the pollen viability contrary to the pollen germination and

pollen tube length (Table 1). Additionally, pollen viability was not significantly correlated with gamma doses (r = 0.319) (Table 2).

4. DISCUSSION AND CONCLUSION

One of the most distinctive features of pollen grains is high radioresistancy. Very high radiation doses are required to inhibit pollen germination and pollen tube growth. Variation in LD50 values of irradiated pollen from a number of species ranged between 130 krad to 220 krad (Speranza *et al*., 1982; Marcucci *et al*., 1984), and pollen grains were almost unable to germinate after 600 krad irradiation (Calzoni and Seperanza, 1982; Tuyl *et al*., 1982; Georgieva and Atanosova, 1986). The process of pollen germination is not one of nuclear and cell division but one of cell enlargement. Therefore, the high levels of ionizing irradiation required to inhibit pollen germination are comparable to those necessary to inhibit cell enlargement in the absence of division.

On the other hand, the irradiation treatments applied at low doses were more effective than at high doses for stimulating pollen germination and pollen tube growth in some species (Aly, 1995; Banerji *et al*., 1996).

Our data on stimulating apricot pollen germination at low doses are in agreement with these studies. After pollen irradiated at range of 25-50 krad , the pollen germination decreased in comparison with the control (Table 1).

Particularly, it appears that both metabolic and physical factors are involved in the observed pollen germination and pollen tube elongation following radiation. Metabolic factors may be more prominent at low and modarate doses, physical factors more prominent at high doses (Visser and Oost, 1981). This fact suggests that protein synthesis is crucia. And also, enzymes are relatively radioresistant molecules, in fact, that their activity can be stimulated by irradiation. According to Georgieva and Atanassov (1986), high doses of gamma irradiation inhibited pollen germination and pollen tube growth but did not affect or slightly decreased the activity of glutamate dehydrogenase (GDH), isocytrate (IDH), malate dehydrogenase (MDH), glucose-6-phosphate dehydrogenase (G6PDH) and acid phosphatase. The radioresistance of the activity of

these enzymes appears to be responsible for the high radioresistance of pollen.

The results of our investigation on pollen viability are in agreement with Zottini *et al*. (1997). In our study, contrary to results from pollen germination and pollen tube length, the pollen viability was stimulated with high doses of gamma. These differences may be due to the effect of irradiation on different enzymes activity. Calzoni and Sprenza (1982) observed that some of the enzymes (acid phosphatase, ribonuclease etc) were increased, while others were unaffected by irradiation.

Our trials failed to achieve a stimulating effect in Sakı apple pollen tube growth by irradiated with low gamma doses .There was a weak stimulating effect determined in pollen tube growth after irradiated with 5 and 10 krad gamma doses. However, pollen tube length decreased seriously when the irradiation dose in increased up to 20 krad. Similar results were obtained by Marcucci *et al*. (1984) for Golden Delicious apple pollen. High doses of irradiation inhibited the lactate dehydrogenase (LDH) and alchol dehydrogenase (ADH) activity which take part in the anaerobic braekdown of carbohydrates as well as the AS-D-esterase (pectin esterase) and β-glucosidase activity which are involved in the cell wall metabolism. It was concluded that the gamma irradiation intibited the process of pollen tube elongation through inactivation of the enzymes AS-D- esterase and β-glucosidase which hydrolased and softened the cell wall (Georgieva and Atanassov, 1986).

Our findings illustrate that there was a slight stimulation effect on pollen germination and pollen tube length with low irradiation doses. The negative effects of high doses gamma irradiation were similar on both pollen germination and pollen tube length. We can conclude that although the biological effects of large doses of ionizing radiation are harmful, low to intermediate doses are enhance some biological factors in apple pollen.

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