African Journal of Biotechnology Vol. 8 (24), pp. 6918-6926, 15 December, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Influence of gamma irradiation on pollen viability, germination ability, and fruit and seed-set of pumpkin and winter squash

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Accepted 6 November, 2009

The influence of irradiation dose (50, 100, 200 and 300 Gray), irradiation time (July 9th, 11th, 15th, 21st and 28th) and pollen age (0th and 1st days) on the pollen viability, germination ability and fruit and seed-set were investigated in pumpkin (*Cucurbita moschata* Duchesne ex Poir.) and winter squash (*Cucurbita maxima* Duchesne ex Lam.). Along with increasing of irradiation dose, irradiation period and pollen age, pollen viability, germinability and fruit and seed-set decreased, significantly. Irradiated and non-irradiated pollens maintained their viability for 1 - 4 days and 4 - 7 days, respectively. Non-irradiated pollens of winter squash were germinated for 2 days and produced the highest germination rate (22.6 and 22.9%) on July 9th and 11th, respectively. Moreover, irradiated pollens were germinated for 2 days at 50 Gray (1.1 - 8.5%) and for one day at 100 Gray (0.6 - 10.4%). On July 9, the fruit-set rate ranged from 75.0% (at 50 Gray) to 63.0% (at 100 Gray) by pollination with 0th day pollens in winter squash. Pollination with irradiated pollens at 50 Gray and 100 Gray gave both seeded and seedless fruits depending on irradiation periods. All fruits obtained from pollinations with non-irradiated pollens were seeded. Although irradiated pollens at 200 Gray and 300 Gray were not germinated in medium, they gave fruit more or less, but fruits were seedless. The highest fruit-set rates were determined 75.0% (57SI21) in winter squash and 26.3 (55BA01) in pumpkin, pollination with 0th day old pollens. In all irradiation times, the percentage of fruit and seed-set of pumpkin was lower than that of winter squash.

Key words: Gamma irradiation, pollen viability and germination ability, fruit and seed-set, pumpkin, winter squash.

INTRODUCTION

Irradiated pollen technique (UV, gamma rays and X-rays) is currently used to induce *in situ* haploid plants. In this technique, pollens irradiated with relatively higher irradiation doses. Irradiated pollen is genetically inert, but physiologically active and germinates on the stigma, but it cannot fertilize the egg-cell and the polar nuclei. These pollens might be used to stimulate parthenogenesis, including gynogenic haploid production; overcoming minor cross-incompatibilities and physiological studies of incompatibility (Stairs and Mergen, 1964; Savaşkan and Toker, 1991; Todorova et al., 2004), gene transformation (Pandey, 1978) and nucleus substitution (Raquin et al., 1989).

Gamma rays are commonly used in haploidy programmes because of their simple application, good penetration, reproducibility, high mutation frequency and less disposal problems (Chahal and Gosal, 2002). Induction of gynogenic haploid embryo and obtaining plants have been achieved through irradiated pollen technique in melon (Sauton and Dumas de Vaulx, 1987; Cuny, 1992; Maestro-Tejada, 1992; Sari et al., 1992; Abak et al., 1996), cucumber (Truong-Andre, 1988; Sauton, 1989; Niemirowicz-Szczytt and Dumas de Vaulx, 1989; Çağlar and Abak, 1999), watermelon (Gürsöz et al., 1991; Sarı, 1994), snake cucumber (Yanmaz et al., 1999) and squash (Kurtar et al., 2002). Pollen irradiation was proved to be effective for haploid induction but the embryo yield was found to be highly influenced by different factors such as genotype, environmental conditions, irradiation dose (Ficcadenti et al., 1995), size and shape of the pollen grain and the thickness of the pollen wall (Giles and Prakash, 1987).

Pumpkin and winter squash are monoecious and have bright yellow and light orange colour of the petals and the campanula shape of the flowers attractive for insect (bees are major pollinators) pollination. They have the largest pollen in cucurbits (approximately 180 μ) as in squash (*Cucurbita pepo* L.). Pollen exposure and availability to insect visitors is within the period that the flowers are open. Although the male flowers appear earlier than the female ones, the peaks of flowering in both sexes almost overlapped, favouring female recaptivity (Nepi and Pacini, 1993; Ikechukwu et al., 2007). Pollen grains of pumpkin and winter squash were highly sensitive to dehydration and loss their viability rapidly as in pollen grains of squash (Kerhoas et al., 1986; Nepi and Pacini, 1993). Therefore, studies on pollen viability and germination ability and fruit and seed-set are the most reliable factors determining the appropriate irradiation dose, irradiation period and pollen age for haploid embryo induction.

The objective of the present study was to determine the effects of irradiation dose (Co^{60}), irradiation time and pollen age on pollen viability, germination ability and fruit and seed-set in pumpkin and winter squash.

MATERIALS AND METHODS

Materials

Six winter squash genotypes (57SI06, 57SI21, 55BA02, 55BA03, 55ÇA06 and G14) and five pumpkin genotypes (55BA01, 14YE01, 14YE02, 14BO01 and G9) were used as plant materials. Genotypes were selected from the Black Sea region of Turkey (genetic material used in the project was funded by TUBITAK-TOVAG - 104O144), except “G14” and “G9” genotypes which were provided by the Turkish Seed Gene Bank, Menemen, Izmir.

Irradiation and pollen obtention

Female flowers were isolated with white cloth bags (15 x 10 cm) and male flowers were collected around noon on the day before anthesis. Anthers without filaments were collected and mixed equally for each genotype. Irradiation was performed in small cardboard boxes (5 x 7 x 2 cm) by Cobalt 60 gamma-rays at doses of 50, 100, 200 and 300 Gray (1 Gray = 100 Rad). To evaluate the effect of vegetation period on influence of pollen viability, germination ability and fruit and seed-set, anthers were irradiated at different times (July 9th, 11th, 15th, 21st and 28th). Irradiated anthers were incubated at room temperature overnight. On the morning of the following day (designated as 0th day), pollens were placed in petri dishes carefully and stored in a refrigerator at 4°C thoroughly tests.

Viability and germination ability tests

Non-irradiated and irradiated pollens were placed to three areas on glass slides and stained with a drop of 1% TTC (2, 3, 5 - Triphenyl Tetrazolium Chloride) solution (Stanley and Linskens, 1974). Stained pollens were placed at room temperature (25 ± 1°C). Three viability classes were defined; 1. Pollen grains with bright red stain (viable), 2. Pollen grains with pink stain (low-viable), 3. Pollen grains with unstained (non-viable). Brewbaker and Kwack (1963) media (20% sucrose, 100 mg l^{-1} H_3BO_3, 300 mg l^{-1} $Ca(NO_3)_2.4H_2O$, 200 mg l^{-1} $MgSO_4.7H_2O$ and 100 mg l^{-1} KNO_3 in 0.2% agar solution) was used to study *in vitro* germination ability of non-irradiated and irradiated pollens by sitting drop culture method (Zaman, 2006). The medium was dropped in dishes and pollens were sprinkled on the media by gently and petri dishes closed to prevent water loss of pollens. Approximately, 400 - 500 pollen grains were sprinkled on each petri dish. The petri dishes were incubated under desired temperature (25°C) in a test cabinet.

Pollen viability and germination ability were observed by direct microscopy (Nikon Alphaphot-2 YS2, Japan). Approximately 300 pollen grains were examined and counted after 4 h for viability tests and 24 h for germination ability tests of setting the experiment (Şensoy et al., 2003). A pollen grain with bright red was considered viable (Figure 1). Pollen was accepted germinated when pollen tube length was at least equal to or greater than the grain diameter (Figure 2). Three glass slides and petri dishes were used as replications for viability and germination ability tests, respectively. Response to viability and germination ability was expressed in percentage (%).

Pollination, fruit and seed-set

On the morning (between 7$^{.00}$ - 9$^{.00}$ a.m.) of the following day (0th day), female flowers were pollinated with irradiated pollens at 0th and 1st days for each irradiation dose, irradiation time and genotypes. Female flowers were then isolated with cloth bags again to prevent undesired pollen contamination. Cloth bags were removed at 2nd or 3rd days of pollination. Fruit-set was determined in harvest periods and seed-set was studied as seeded or seedless during extraction. Fruit and seed-set were evaluated and expressed as percentage (%).

RESULTS

Effects of irradiation dose, irradiation time and pollen age on pollen viability

The pollen viability was affected from irradiation doses, irradiation time, pollen ages and species. Along with the increasing doses of gamma ray, irradiation period and pollen age, pollen viability (PV) continuously decreased as compared to non-irradiated pollens (Figures 3 and 4). On July 9th, PV was the highest (92.2%) in winter squash at 50 Gray and the lowest (14.7%) in pumpkin at 300 Gray at the 0th day. The highest PV was recorded in non-irradiated pollens of winter squash (98.4%) and pumpkin (91.8%). Pollen viability ended at 200 and 300 Gray and stained pollen was not found at the 2nd day for pumpkin and at the 3rd and 2nd day for winter squash, respectively. Viability of irradiated pollens ranged from 4 days (at 50 Gray) and 2 day (at 100 Gray) while non-irradiated pollens were viable for 4-7 days. Besides, on July 21st and 28th, PV was reduced sharply and non-irradiated pollens were viable 81.4 and 61.9% in winter squash and 69.5 and 45.3% in pumpkin at the 0th day, respectively. Similarly, the PV of irradiated pollens at 50 Gray and 100 Gray was counted 53.7 and 31.7% in winter squash and 40.6 and 23.3% in pumpkin on July 28th. Viable pollen was not recorded at 300 Gray dose at the 1st day and at 200 Gray at the 2nd day on July 21st and 28th. PV of winter squash was found higher than that of pumpkin.

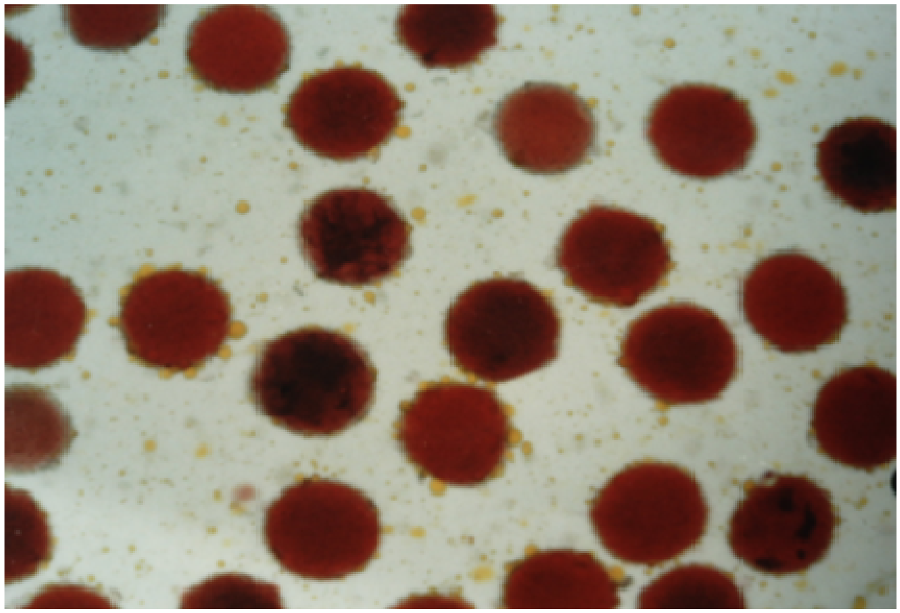

Figure 1. Stained pollens with 1% TTC solution.

Figure 2. Germinated pollens in medium.

Effects of irradiation dose, irradiation time and pollen age on pollen germinability

Germination percentage (GP) was influenced highly from irradiation dose, irradiation time, pollen age and species (Figure 5). *In vitro* GP of winter squash and pumpkin pollens was found lower both of irradiated and non-irradiated pollens. Pollen grains were germinable only at the 0^{th} and 1^{st} days. Moreover, irradiated pollens at 200 and 300 Gray were not germinated in medium at all periods. On July 9^{th}, at the 0^{th} day, GP was found 18.4 and 10.4% in winter squash and 14.6 and 6.8% in pumpkin at 50 Gray and 100 Gray, respectively. GP were decreased sharply at the 1^{st} day and ended at the 2^{nd} day.

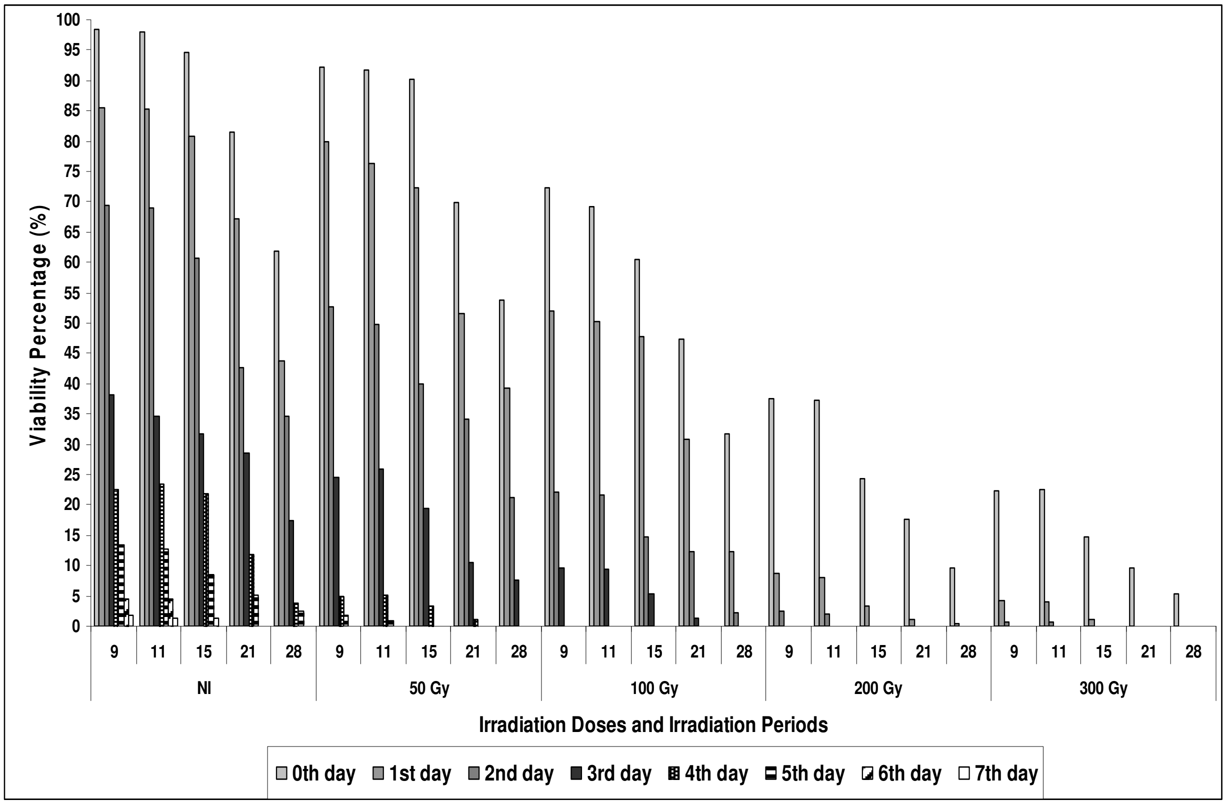

Figure 3. Influence of irradiation doses, irradiation times and pollen age on pollen viability (%) in winter squash. (NI: Non-irradiated) Error bars indicate standard error of the means (P<0.05).

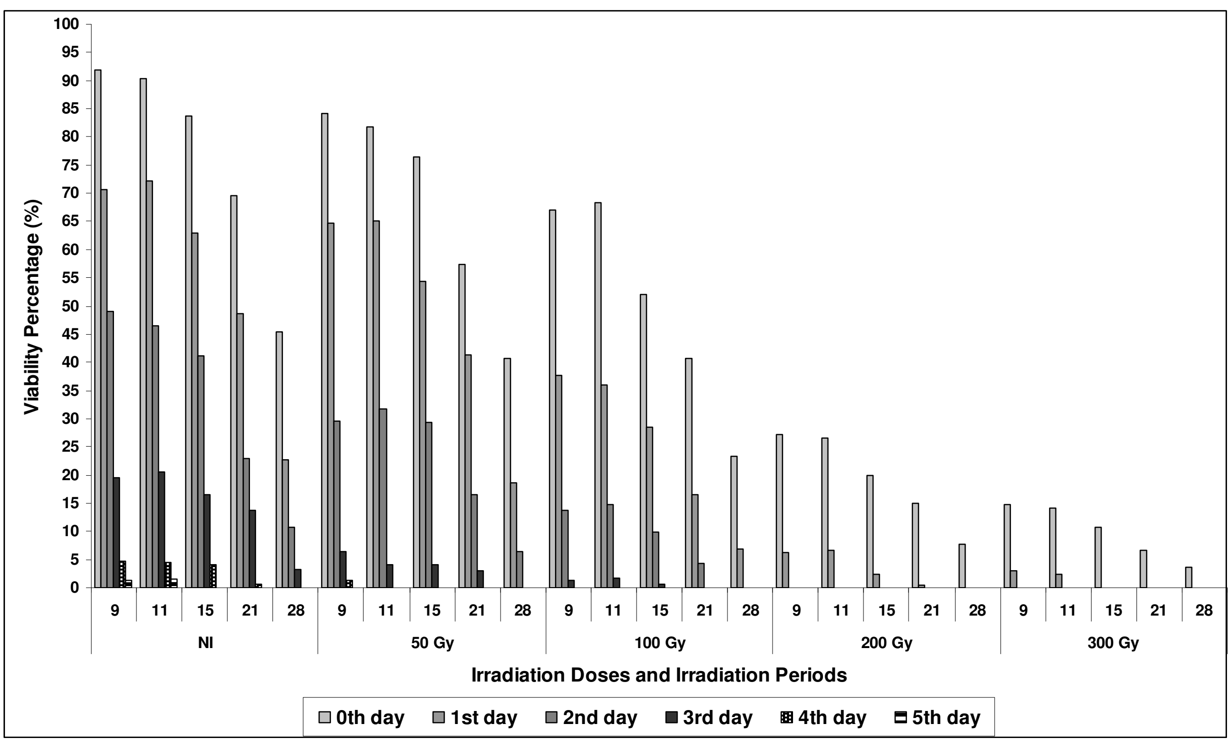

Figure 4. Influence of irradiation doses, irradiation times and pollen age on pollen viability (%) in pumpkin. (NI: Non-irradiated). Error bars indicate standard error of the means (P<0.05).

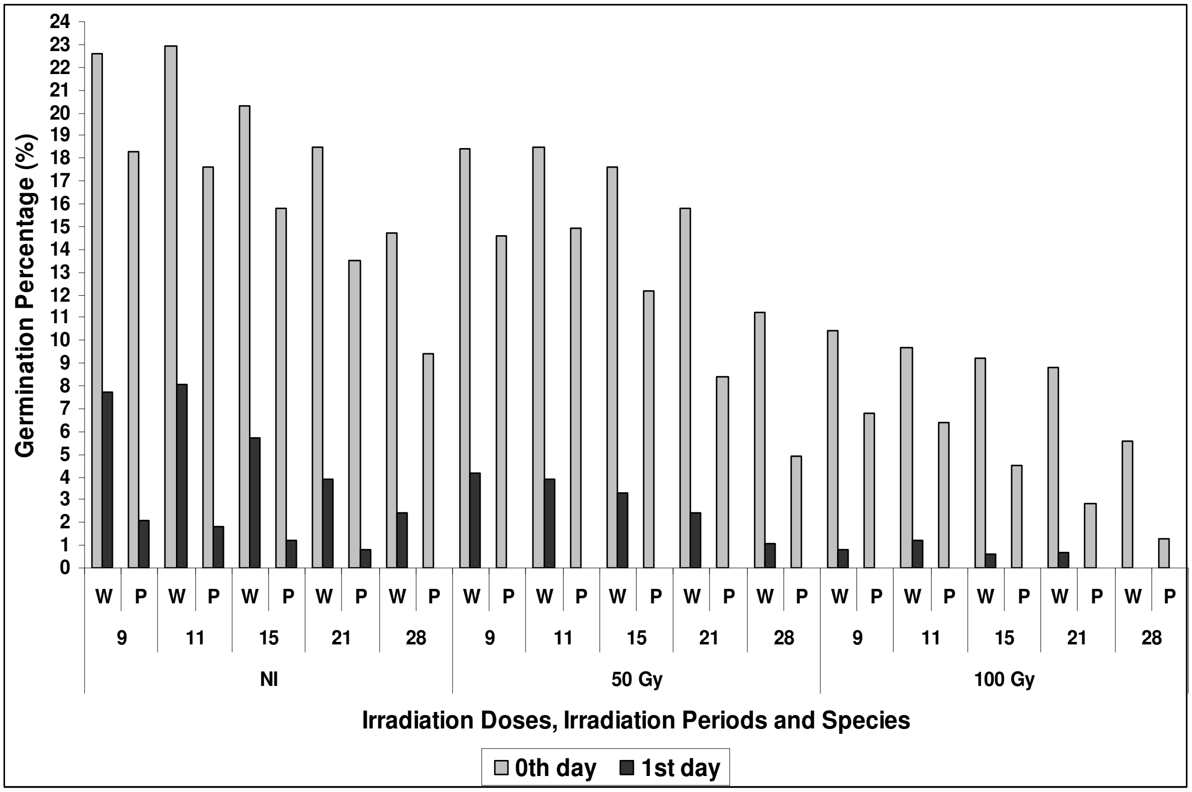

Figure 5. Influence of irradiation doses, irradiation times and pollen age on pollen germination ability (%) in winter squash and pumpkin. (NI: Non- irradiated; W: Winter squash; P: Pumpkin). Error bars indicate standard error of the means (P<0.05).

GP of non-irradiated pollens were recorded 22.6% in winter squash and 18.3% in pumpkin at the 0^{th} day. On July 21^{st} and 28^{th}, GP were recorded 15.8 and 11.2% in winter squash and 8.4 and 4.9% in pumpkin at 50 Gray, respectively. GP of pumpkin pollens were lower than that of winter squash.

Effects of irradiation dose, irradiation time, pollen age and genotypes on fruit and seed-set

Fruit-set rate (FR) and seed-set rate (SR) were significantly influenced of irradiation dose, irradiation time, pollen age and genotypes (Figures 6, 7 and Table 1). Along with the increasing dose of gamma ray, irradiation time and pollen age FR decreased and seedless fruit increased. While non-irradiated pollens gave the fruit-set with seeded in all periods, fruit and seed set were obtained only pollination with 50 Gray and 100 Gray. Irradiated pollens at 200 Gray and 300 Gray gave fruit-set more or less, but fruits were seedless. At the 0^{th} day, FR was counted as 75% in winter squash and 50% in pumpkin at 50 Gray and all fruits were seeded on July 9^{th}. The highest FR was 88% in non-irradiated pollens and the lowest FR was determined 13% in winter squash and in pumpkin at 300 Gray. At the 1^{st} day, only winter squash had fruit-set with seedless at 50 Gray (25%) and 100 Gray (13%). Furthermore, irradiated pollens with 50 Gray and 100 Gray gave seedless fruits more or less on July 21^{st} and 28^{th}. In this respect, seedless fruits were determined 25 and 50% at 50 Gray and all fruits were seedless at 100 Gray on July 21^{st} and 28^{th}, respectively.

FR and SR were influenced by investigated genotypes and the highest FR was determined 91.7 (57SI21) in winter squash and 26.3 (55BA01) in pumpkin, pollination with 0^{th} day old pollens. In contrast, G14 (22.7%) and 14YE02 (5%) had the lowest FR. At 1^{st} day, FS considerably reduced and SF was not performed in winter squash and both FS and SF were not determined in pumpkin. At 1^{st} day, FS was found 9.3 and 6.3% in winter squash and pumpkin, respectively. Winter squash genotypes had higher FR and SR values than that of pumpkin genotypes.

DISCUSSION

Along with increasing doses of gamma-ray, irradiation time and pollen age, pollen viability, germination ability, fruit and seed-set decreased significantly and this decrease was more rapidly than non-irradiated pollens. These findings were also in accordance with those reported in many

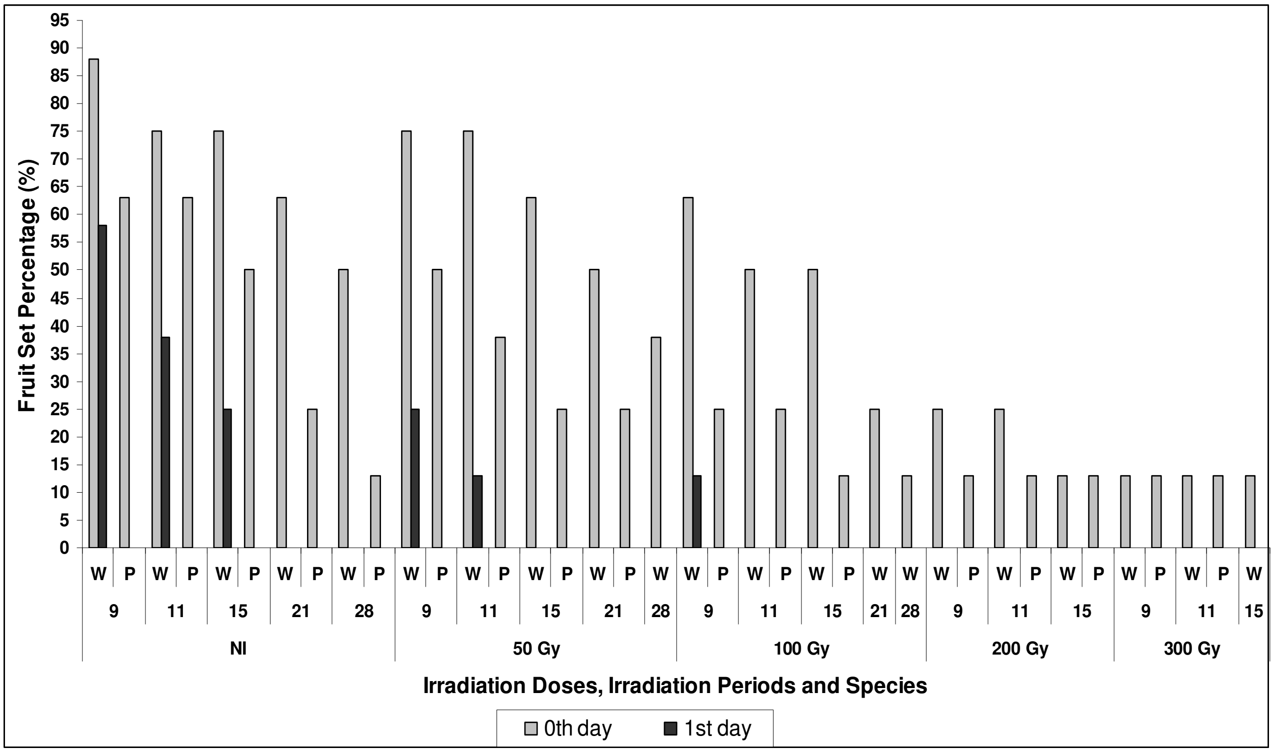

Figure 6. Influence of irradiation doses, irradiation times and pollen age on fruit-set (%) in winter squash and pumpkin. (W: Winter squash; P: Pumpkin; NI: Non-irradiated).Error bars indicate standard error of the means (P<0.05).

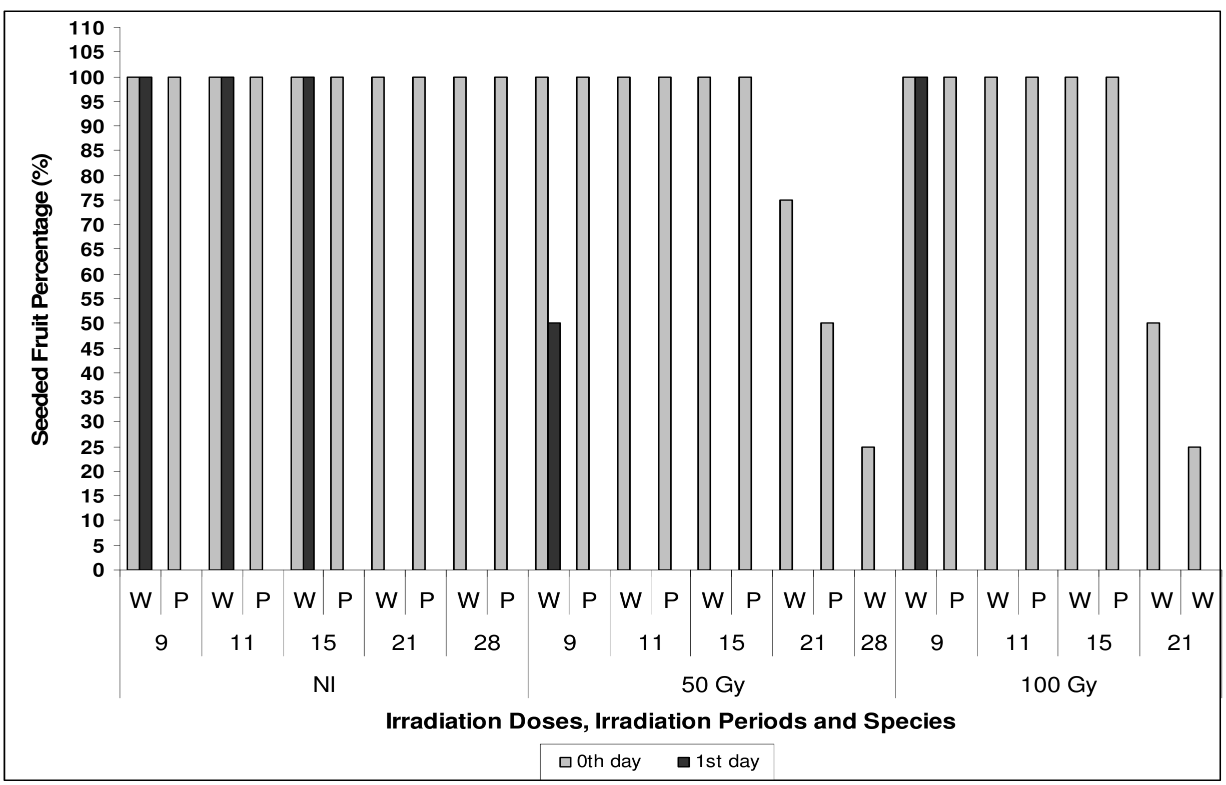

Figure 7. Influence of irradiation doses, irradiation times and pollen age on seeded fruit (%) in winter squash and pumpkin. (W: Winter squash; P: Pumpkin; NI: Non-irradiated). Error bars indicate standard error of the means (P<0.05).

Table 1. Effects of pollen age on fruit-set rate (FR) (%) and seeded fruit rate (SR) in winter squash and pumpkin genotypes.

Genotypes		0^{th} day					1^{st} day					Overall				
		PF	FS	FR	SF	SR	PF	FS	FR	SF	SR	PF	FS	FR	SF	SR
W S	57SI06	17	7	41.2	7	100	12	1	8.3	0	0	29	8	27.6	7	87.5
	57SI21	24	22	91.7	18	81.8	14	2	14.3	0	0	38	24	63.2	18	75
	55BA02	21	9	42.9	7	77.8	8	0	0	0	0	29	9	31.0	7	77.8
	55BA03	19	7	36.8	7	100	6	1	16.7	0	0	25	8	32.0	7	87.5
	55ÇA06	19	6	31.6	6	100	8	1	12.5	0	0	27	7	25.9	6	85.7
	G14	22	5	22.7	5	100	6	0	0	0	0	28	5	17.9	5	100
Σ		122	54	44.3	50	92.6	54	5	9.3	0	0	176	59	33.5	50	84.7
P	55BA01	19	5	26.3	4	80	7	0	0	0	0	26	5	19.2	4	80
	14YE01	21	5	23.8	4	80	4	0	0	0	0	25	5	20.0	4	80
	14YE02	20	1	5.0	1	100	4	0	0	0	0	24	1	4.2	1	100
	14BO01	18	4	22.2	2	50	6	0	0	0	0	24	4	16.7	2	50
	G9	17	3	17.7	2	66.7	5	0	0	0	0	22	3	13.6	2	66.7
Σ		95	18	19.0	13	72.2	26	0	0	0	0	121	18	14.9	13	72.2
Overall		217	72	33.2	63	87.5	80	5	6.3	0	0	297	77	25.9	63	81.8

W: Winter squash; P: Pumpkin; PF: Pollinated flower number; FS: Fruit number; SF: Seeded fruit number.

researchers, such as Van Den Boom and Den Nijs (1983) in gherkins, Denissen and Den Nijs (1987) in cucumber, Dore (1989) in white head cabbage, Pandey et al. (1990) in kiwifruit, Cuny and Roudot (1991) and Sarı et al. (1992) in melon, Cuny (1992), Falque et al. (1992) in cocoa, Sari (1994) in watermelon, Abak et al. (1997) in some cucurbit crops, Yanmaz et al. (1999) in snake cucumber, Kurtar et al., (2002) in squash and Kumar and Priyanka (2006) in soybean reported that pollen viability, germination ability and fruit-set decreased at high doses gamma irradiation depend on genotypes and irradiation doses.

While irradiated pollens maintained their viability 1 - 4 days, non-irradiated pollens were viable for 4 - 7 days under *in vitro* conditions. At the 0^{th} day, germination percentages were found to be 18.4 and 10.4% in winter squash and 14.6 and 6.8% in pumpkin at 50 Gray and 100 Gray, respectively on July 9^{th}. High irradiation doses had lethal effects and pollen grains did not germinate at 200 and 300 Gray. The lowest pollen viability and germinability were determined on July 21^{st} and 28^{th}. The results indicated that, pollen viability and germinability were significantly lower in winter squash and pumpkin. In similarly, Nepi and Pacini (1993) and Ikechukwu et al. (2007) reported that pollen viability is about 90 - 96% in newly opened flowers but decreases to about 62 - 78% on closure and crashes to 8 - 10% after a day in squash under field conditions. On the other hand pollens of winter squash and pumpkin were found to be sensitive to irradiation due to being the largest pollen in vegetables because of radio-resistance decreased as pollen diameter increased (Brewbaker and Emery, 1962; Alison and Casareft, 1968; Shridhar, 1992).

Pollen morphology and biology of winter squash, pumpkin and squash are similar. They have lower pollen viability and germinability related to both high sensitivity to dehydration (Kerhoas et al., 1986) and irradiation (Kurtar et al., 2002) due to having many apertures (Şensoy et al., 2003). Partially hydpercentaged pollen especially that with low sucrose and polysaccharide content, is not able to control water content because of its reduced possibility to interconvert carbohydpercentages and to increase turgor pressure. They quickly lose their water and die (Kerhoas et al., 1986; Nepi et al., 2001; Franchi et al., 2002). Therefore, pollen viability, germinability and fruit-set of winter squash and pumpkin highly decreased as reported in squash, results of abnormal meiosis forming or unequal gametes. The structure and physiology of the pollen grains is under genetic control and irregular or abnormal meiosis may cause significant changes in the pollen properties (Nepi and Pacini, 1993). The failure of pollen germination at first and second day is associated with both of dehydration and irradiation.

Fruit and seed-set were affected on irradiation dose, irradiation time, pollen age and genotypes. Along with increasing irradiation dose, irradiation time and pollen age fruit-set was reduced and fruits were entirely seedless (parthenocarpic). Irradiated pollens at higher gamma-ray doses (200 Gray and 300 Gray) had confusingly fruit-set, but fruits were seedless. This result supported to tendency to parthenocarpy in winter squash and pumpkin and accordance with our previous work on summer squash (Kurtar, 2003). Cicero et al. (2005) reported that parthenocarpy is controlled by a single locus, with incomplete dominance in the direction of parthenocarpic expression in squash.

In addition, pollen viability, germination ability, fruit and seed set were affected from irradiation time. On July 21^{st} and 28^{th}, along with the decreasing percentage of viable and germinable pollen, fruit-set and seed development was reduced sharply. Moreover, fruit and seed-set were influenced by investigated genotypes due to different parthenocarpic reaction. Winter squash genotypes had higher fruit and seed-set values than that of pumpkin genotypes, because of having longer pollen viability and higher germination ability response.

Finally, pollen viability and germination ability and fruit and seed set significantly decreased at higher doses gamma rays (200 Gray and 300 Gray), delayed pollination periods (on July 21st and 28th) and aged pollen (at the 1st day). In addition, pollen viability test gave a rough estimation because of immature and unviable grains may also get stained and this may cause an increase on pollen viability. On the other hand pollen germination test gave more suitable estimation in winter squash and pumpkin. But, germination test must be improved to found real germination ability in winter squash and pumpkin.

Conclusion

In conclusion, to ensure of (gynogenic) gynogenic haploid embryo induction in winter squash and pumpkin pollens should be irradiated with low doses gamma rays (> 200 Gray) and female flowers should be pollinated as early as possible in a day, while the pollen is still germinable.

ACKNOWLEDGEMENTS

We gratefully acknowledge the support of the Scientific and Technical Research Council of Turkey (TUBİTAK Project No: TOVAG 108 O 390) and partial funding by the Vocational School of Bafra, Ondokuz Mayis University in Turkey. And, thanks to Tenzile OFLUOGLU and Nilgun Ozbek OKUMUS for irradiation experiments.

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