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Effects of different genotypes and gamma ray doses on haploidization using irradiated pollen technique in squash

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Abstract: Fourteen genotypes and 3 different gamma ray doses were tested to develop an efficient haploidization protocol in squash. For this purpose, male flowers collected 1 day before anthesis were irradiated with 150, 200, and 300 Gy gamma ray doses, and female flowers were pollinated with the irradiated pollens the next day. In the first year of the study, 1858 embryos were obtained from 219 fruits. While 1358 of these were found in fruits irradiated with a 150 Gy gamma dose, the remaining 500 embryos were found in fruits irradiated with 200 Gy. From fruits irradiated with 150 and 200 Gy gamma doses, 9.12 and 3.53 haploid embryos per 100 seeds were obtained, respectively. While Genotype 3 was the most successful genotype with 12.42 embryos per 100 seeds, the minimum embryo numbers were obtained from Genotype 4 with 1.46 embryos per 100 seeds. In the second experimental year, 8 genotypes and the same gamma doses were used, and 2625 haploid and 1378 diploid embryos were obtained from 217 fruits. At irradiation doses of 150, 200, and 300 Gy, 2010, 539, and 76 haploid embryos were found, respectively. Genotype 6 was the most successful genotype with 13.35 embryos per 100 seeds.

Key words: *Cucurbita pepo*, haploid embryo, irradiation dose

1. Introduction

Haploid plants have the same number of chromosomes both in somatic cells and gametic cells. They only contain a series of alleles in each locus, and for this reason haploid plants are very important in breeding studies. The major advantage of haploidy techniques is that they can provide fully homozygous lines in a short time. Homozygous lines can also be obtained via conventional methods. However, this process takes 10–12 years in open-pollinated plants and 6–7 years in self-pollinated plants. This period can be shortened by 1–2 years by applying haploidy techniques. There are 3 haploidy techniques: androgenesis (anther culture, microspore culture), gynogenesis (ovule and ovarium culture), and parthenogenesis (irradiated pollination technique). Anther culture, ovule culture, and ovarium culture studies in different species of the family Cucurbitaceae have been performed (Gémesné and Venczel, 1996; Metwally et al., 1998a, 1998b; Mohamed and Refaei, 2004). However, haploid embryos could not be obtained via anther culture, and only a few haploid embryos were observed in ovule and ovarium culture. Later, researchers focused on parthenogenesis, and the first haploid embryos were found in melon. This technique was used in different Cucurbitaceae species: melon (Gursoz et al., 1991; Cuny et al., 1993; Godbole and Murthy, 2012a, 2012b; Baktemur et al., 2013), watermelon (Sarı et al., 1994; Taşkın et al., 2013), cucumber (Faris et al., 1999; Nikolova and Alexandrova, 2001; Claveria et al., 2005; Chun et al., 2006), and squash. Different researchers have studied this technique in squash to develop a protocol.

Irradiation doses and genotypes (Başay and Ellialtıoğlu, 2013; Olszewska et al., 2014) are 2 of the main factors affecting haploid success. Sandı (1998) used anther culture and irradiated pollination techniques to obtain haploid embryos in squash. Two different doses of cesium (300 and 350 Gy) were used as the irradiation source. Kurtar et al. (2002) examined effects of genotypes (Eskenderany F1, Acceste F1, Sakız, and Urfa Yerli) and gamma doses (25, 50, 75, 100, 200, 300, and 400 Gy) on haploid embryo production in squash. Berber (2009) tested different gamma irradiation doses (50, 100, and 150 Gy) and genotypes (15 genotypes) in pumpkin.

Turkey is one of the most important squash-producing countries with 23,000 ha cultivated and 411,942 t of

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production (http://faostat.fao.org/site/567/default.aspx#ancor). Squash is one of the important species in the family Cucurbitaceae. Therefore, universities, the Ministry of Agriculture, and breeding companies in Turkey focus on squash breeding. Two studies from Turkey (Kurtar et al., 2002; Berber, 2009) used different gamma ray doses and genotypes in squash to develop a protocol for producing haploid embryos; however, some points are still unclear. While 50 Gy was found to be the most successful gamma dose by Kurtar et al. (2002), Berber (2009) found that 150 Gy performed better than 50 or 200 Gy. The purpose of this research is to help develop a protocol for the haploidization technique in squash. With this objective, we tested 3 different gamma ray doses, 150, 200, and 300 Gy, on 14 *Cucurbita pepo* genotypes.

2. Materials and methods

This study was conducted in the Horticulture Department, Faculty of Agriculture, Çukurova University, Adana, Turkey, in 2011 and 2012. Fourteen different squash genotypes obtained from the Rijk Zwaan Company were used in this study. In the second experimental year, 8 genotypes selected from these 14 genotypes were tested. The 8 genotypes were selected according embryo number obtained per fruit. Seeds were planted in plugs (peat and perlite, 2:1) at the Horticultural Research and Application Area of Çukurova University. Seedlings were then transferred to a plastic greenhouse with 1 × 1.75 m spacing. Throughout the growing period normal horticultural cultivation practices were implemented. Male flowers were collected the day before anthesis and put into glass petri dishes for irradiation. On the same day, female flowers were closed with pens to prevent open pollination. Irradiation was performed at the Department of Radiation Oncology, Faculty of Medicine, Çukurova University, with gamma rays from Co^{60}. Two different irradiation doses were examined in the first year of the study: 150 Gy and 200 Gy. In the second year of the study, a dose of 300 Gy was added to the experiment. After irradiation, male flowers were kept at room temperature during the night. On the following day, isolated female flowers were pollinated with irradiated pollens, and the pollinated female flowers were closed with pens to prevent pollen contamination until fruit setting (Figure 1).

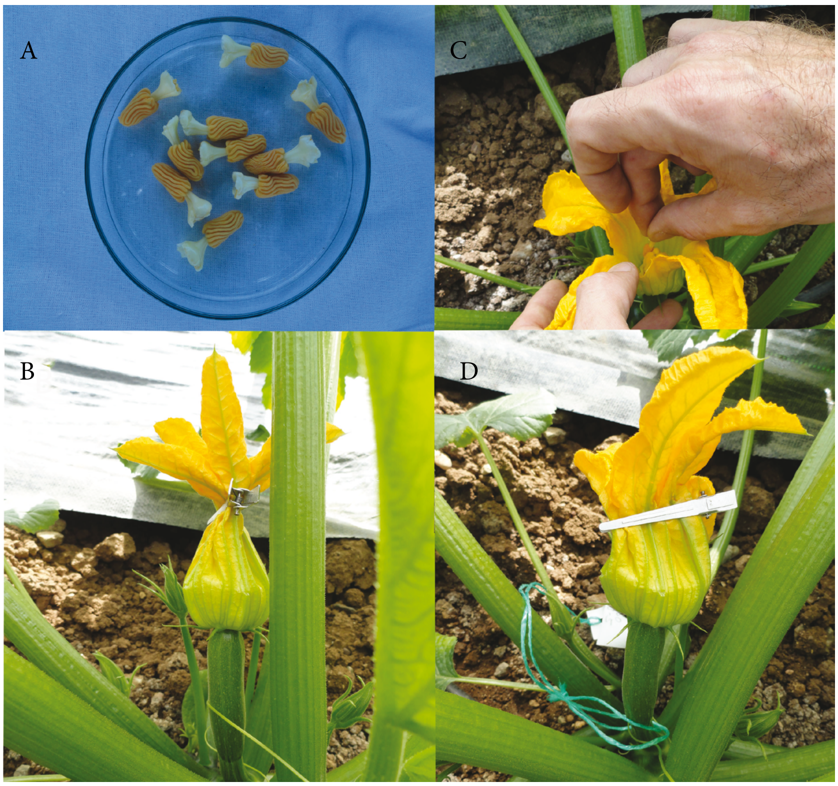

Figure 1. A) Male squash flowers in glass petri dishes for irradiation, B) isolation of female flowers in pens to prevent open pollination, C) pollination of female squash flowers with irradiated pollen of male flowers, D) closing of pollinated female flowers in pens to prevent pollen contamination.

Squash fruits were harvested 35 days after pollination and washed with water for presterilization. After sterilizing via dry burning method with 96% ethanol, the fruits were cut, and seeds were extracted in a laminar flow cabinet. All the seeds were opened one by one under a stereo binocular microscope and checked for embryos. Embryos were placed in glass culture tubes containing nutrient medium. CP medium (Chee et al., 1992) containing 30 g L^{-1} sucrose, 8 g L^{-1} agar, 0.08 mg L^{-1} B12, and 0.02 mg L^{-1} IAA was used as the nutrient medium. Cultured embryos were incubated in a growth chamber at 25 °C under 8 h dark and 16 h light photoperiod conditions. Seed number, haploid embryo number, and diploid embryo number were counted for each genotype and irradiation dose separately. In addition, haploid and diploid embryo numbers per 100 seeds were calculated. Since different types of embryos were observed in the first year of the study, embryos were divided into 6 different groups in the second year of the study (Figure 2).

3. Results

3.1. First experimental year

Two different gamma ray doses were tested in 14 genotypes in order to determine the effects on haploidy. In total, 219 *Cucurbita pepo* fruits were used in this study. While 111 of the fruits were pollinated with pollen irradiated with 150 Gy, the rest (108) were pollinated with pollen irradiated with 200 Gy (Table 1). From 219 fruits, 1858 embryos were obtained. In the fruits irradiated with 150 Gy, 1358 embryos were found, and 500 embryos were found in the fruits irradiated with 200 Gy.

Opened fruit number, haploid embryo number, seed number, and haploid embryo number per 100 seeds in the first year of the experiment are given in Table 2. Fourteen different *Cucurbita pepo* genotypes were used in the study. Genotype is one of the main factors in haploidy studies. The highest embryo number per 100 seeds was observed in Genotypes 3 and 5 (12.42 and 12.20, respectively). The lowest embryo numbers were obtained from Genotype

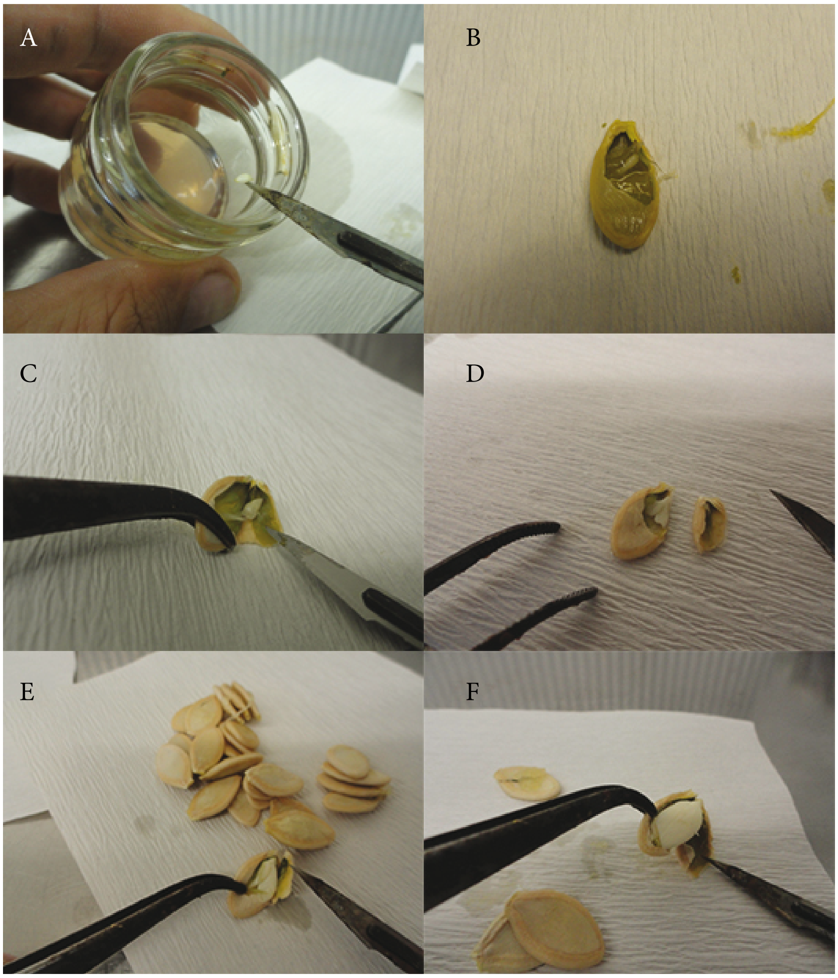

Figure 2. A) Embryo type 1, B) embryo type 2, C) embryo type 3, D) embryo type 4, D) embryo type 5, E) embryo type 6, F) diploid embryo.

Table 1. Opened fruit number, embryo number, and embryo number per fruit in the first year of the experiment.

Genotype	Fruit number		Embryo number		Embryo number per fruit	
	150 Gy	200 Gy	150 Gy	200 Gy	150 Gy	200 Gy
1	4	2	38	6	9.5	3
2	5	2	33	23	6.6	11.5
3	6	6	84	45	14	7.5
4	8	8	80	46	8	5.75
5	9	9	197	59	21.88	6.55
6	5	5	46	14	9.2	2.8
7	8	5	177	24	22.12	4.8
8	9	9	91	52	10.11	5.77
9	10	11	109	28	10.9	2.54
10	9	5	64	6	7.11	1.2
11	5	8	17	4	3.4	0.5
12	12	12	177	43	14.75	3.58
13	11	12	108	100	9.81	8.33
14	10	14	137	50	13.7	3.57
Total	111	108	1358	500	12.23	4.63

Table 2. Opened fruit number, haploid embryo number, seed number, and haploid embryo number per 100 seeds in the first year of the experiment.

G	OFN		HEN		SN		HEN per 100 S		TEN (per genotype)
	150 Gy	200 Gy	150 Gy	200 Gy	150 Gy	200 Gy	150 Gy	200 Gy	Total
1	3	1	11	4	583	86	1.85	4.65	3.25
2	1	3	23	28	292	666	7.87	4.18	6.02
3	2	3	34	37	183	587	18.57	6.28	12.42
4	6	5	55	5	1986	2714	2.74	0.18	1.46
5	3	6	119	37	574	984	20.70	3.71	12.20
6	4	3	39	10	650	314	5.96	3.15	4.55
7	4	4	104	23	941	572	11.05	3.98	7.51
8	6	6	64	49	842	929	7.55	5.23	6.39
9	7	7	82	19	1037	572	7.90	3.30	5.6
10	6	4	39	1	507	324	7.69	0.24	3.96
11	3	5	13	3	212	295	6.09	1.01	3.55
12	9	9	149	67	1661	1519	8.94	4.38	6.66
13	7	8	94	68	873	1343	10.74	5.06	7.9
14	7	10	120	49	1194	1200	10.02	4.08	7.05
Total	68	74	946	400	11,535	12,105	9.12	3.53	

G: Genotype, OFN: opened fruit number, HEN: haploid embryo number, SN: seed number, HEN per 100 S: haploid embryo number per 100 seeds, TEN: total embryo number per genotype.

4 (1.46 embryos per 100 seeds), followed by Genotype 1 with 3.25 embryos per 100 seeds. The number of embryos varied between 3.55 and 7.9 embryos per 100 seeds in other genotypes.

Considering irradiation dose and genotypes together, among the 14 genotypes at 150 Gy, Genotypes 5 and 3 gave the maximum number of haploid embryos with 20.7 and 18.57 embryos per 100 seeds, respectively. Genotype 1 was the most unsuccessful genotype with 1.89 haploid embryos per 100 seeds. At 200 Gy, Genotype 3 was the best with 6.28 embryos per 100 seeds. The lowest number of embryos was obtained from Genotypes 4 and 10 with 0.18 and 0.24 embryos per 100 seeds, respectively.

Seed numbers per fruit varied from 59 to 542.8. At a 150 Gy irradiation dose, Genotype 11 had the fewest seeds, with 70.6 seeds per fruit, and contained 4.3 haploid embryos per fruit. In addition, Genotype 10 contained 84.5 seeds per fruit with 6.5 haploid embryos per fruit. Genotype 4 had the highest seed number, but only 9.2 embryos per fruit were obtained. Although Genotype 5 had fewer seeds (191.3 seeds per fruit) than Genotype 4, it gave more embryos, with 39.6 embryos per fruit. At a 200 Gy irradiation dose, the highest seed numbers were obtained from Genotype 4, but only 1 embryo could be found. The highest embryo number was observed in Genotype 3 with 12.33 embryos per fruit.

3.2. Second experimental year

In the second year of the experiment, 3 different gamma doses (150, 200, and 300 Gy) and 8 genotypes were used, and 217 fruits pollinated with irradiated pollen opened; 2625 haploid and 1378 diploid embryos were obtained from these fruits. From 150, 200, and 300 Gy gamma treatments, 80, 71, and 66 fruits were obtained, respectively. While 2010 embryos of a total of 2625 haploid embryos were obtained from a 150 Gy gamma dose, 200 and 300 Gy doses resulted in 539 and 76 haploid embryos, respectively.

Opened fruit number, diploid embryo number, seed number, diploid embryo number per 100 seeds, haploid embryo number per 100 seeds, and haploid embryo number – haploid embryo number per 100 seeds in terms of embryo types in the second year of the experiment are given in Tables 3 and 4 according to irradiation doses and genotypes. Among the 3 gamma doses tested, the highest number of embryos was obtained from a 150 Gy gamma dose (18.68 haploid embryos per 100 seeds), while 9.03 embryos per 100 seeds were found at a 200 Gy gamma dose. The 300 Gy dose resulted in 1.51 haploid embryos per 100 seeds. From the embryo types numbered 1–4, an average of 3.54, 1.94, and 0.31 embryos per 100 seeds were obtained in 150, 200, and 300 Gy gamma doses, respectively. From embryo types numbered 5 or 6, an average of 2.27, 0.64, and 0.14 embryos per 100 seeds were obtained at 150, 200, and 300 Gy gamma doses, respectively.

The results of genotype effects on haploid embryo formation are given in Table 4. According to Table 4, the highest embryo number was obtained from Genotype 6 with 13.35 embryos per 100 seeds among 8 tested genotypes. It was followed by Genotype 4 (11.63 embryos per 100 seeds), Genotype 2 (11.22 embryos per 100 seeds), Genotype 8 (10.1 embryos per 100 seeds), Genotype 1 (9.29 embryos per 100 seeds), Genotype 3 (8.03 embryos per 100 seeds), Genotype 7 (7.38 embryos per 100 seeds), and Genotype 5 (6.90 embryos per 100 seeds). In terms of embryo type, 1.81 embryos per 100 seeds, 2.46 embryos per 100 seeds, 1.49 embryos per 100 seeds, 2.2 embryos per 100 seeds, 1.29 embryos per 100 seeds, 2.56 embryos per 100 seeds, 1.47 embryos per 100 seeds, and 2.13 embryos per 100 seeds were obtained from embryo types 1–4 in Genotypes 1–8, respectively. In addition, 1.02 embryos per 100 seeds, 0.72 embryos per 100 seeds, 1.03 embryos per 100 seeds, 1.41 embryos per 100 seeds, 0.85 embryos per 100 seeds, 1.54 embryos per 100 seeds, and 0.8 embryos per 100 seeds were obtained from embryo types 5 and 6 in Genotypes 1–8, respectively.

When irradiation dose and genotype are considered together, Genotype 6 and 150 Gy gamma dose were the most successful genotype and irradiation dose with 25.77 embryos per 100 seeds. This was followed by Genotype 8 and 150 Gy gamma dose (24.90 embryos per 100 seeds) and Genotype 2 and 150 Gy gamma dose (21.91 embryos per 100 seeds). On the other hand, Genotype 8 and 300 Gy gamma dose gave the lowest number of embryos with 0.30 embryos per 100 seeds. While Genotype 8 gave the maximum number of embryos per 150 Gy gamma dose, the minimum number of embryos was obtained from the same genotype at the 300 Gy gamma dose. Genotype 8 and 300 Gy gamma dose was followed by Genotype 7 and 300 Gy gamma dose, Genotype 5 and 300 Gy gamma dose, and Genotype 6 and 300 gamma dose with 0.80 embryos per 100 seeds, 1.44 embryos per 100 seeds, and 1.65 embryos per 100 seeds, respectively.

Diploid embryo number is just as important as haploid embryo number in haploidy studies. Improving an efficient and successful method for haploidization depends on obtaining high numbers of haploid and low numbers of diploid embryos. Diploid embryo numbers were 11.99 embryos per 100 seeds, 2.53 embryos per 100 seeds, and 0.30 embryos per 100 seeds at 150, 200, and 300 Gy, respectively.

Seed number was affected by irradiation dose. A higher dose of irradiation reduced the number of seeds. While the maximum seed number was observed in 150 Gy gamma dose with 133 seeds per fruit, the minimum seed number was obtained from 300 Gy with 74 seeds per fruits, and a 200 Gy gamma dose gave 92 seeds per fruit.

Table 3. According to irradiation dose: opened fruit number, diploid embryo number, seed number, diploid embryo number per 100 seeds, haploid embryo number per 100 seeds, and haploid embryo number – haploid embryo number per 100 seeds in terms of embryo type in the second year of experiment.

G	ID	OFN	DEN	HEN	SN	DEN 100 S	HEN 100 S	Embryo type HEN-HEN per 100 S #					
#	Gy	#	#	#	#	#	#	1	2	3	4	5	6
1	150	10	149	161	949	15.70	16.96	27 2.84	23 2.42	36 3.79	26 2.74	33 3.48	16 1.69
2	150	10	249	373	1702	14.63	21.91	85 4.99	84 4.93	88 5.17	56 3.29	45 2.64	15 0.89
3	150	10	136	309	1949	6.98	15.85	31 1.59	65 3.34	81 4.15	45 2.31	55 2.82	32 1.64
4	150	10	190	176	1007	18.87	17.47	15 1.49	34 3.38	41 4.07	38 3.77	37 3.67	11 1.09
5	150	10	153	210	1489	10.27	14.10	18 1.21	35 2.35	42 2.82	48 3.22	42 2.82	25 1.68
6	150	10	157	351	1362	11.53	25.77	53 3.89	82 6.02	67 4.92	53 3.89	51 3.74	45 3.31
7	150	10	80	112	898	8.91	12.47	24 2.67	25 2.78	22 2.45	17 1.89	17 1.89	7 0.79
8	150	10	116	318	1277	9.08	24.90	64 5.01	61 4.78	98 7.67	42 3.29	40 3.13	13 1.02
Mean		10	153	251	1329	11.99	18.68	39.6 2.96	51.1 3.75	59.3 4.38	40.6 3.05	40 3.02	20.5 1.51
1	200	10	27	45	516	5.23	8.72	10 1.94	12 2.32	15 2.91	5 0.97	2 0.39	1 0.19
2	200	10	14	79	769	1.82	10.27	25 3.25	28 3.64	15 1.95	7 0.91	4 0.52	0
3	200	10	9	50	784	1.15	6.37	9 1.15	8 1.02	12 1.53	11 1.40	8 1.02	2 0.25
4	200	10	25	111	734	3.40	15.12	16 2.18	17 2.32	30 4.09	23 3.13	23 3.13	2 0.27
5	200	9	20	48	931	2.15	5.15	5 0.54	10 1.07	16 1.72	11 1.18	6 0.64	0 0
6	200	8	19	69	546	3.48	12.63	19 3.48	13 2.38	17 3.11	10 1.83	10 1.83	0 0
7	200	4	14	52	585	2.39	8.88	20 3.41	14 2.39	10 1.71	0 0	6 1.03	2 0.34
8	200	10	11	85	1675	0.65	5.07	21 1.25	17 1.02	18 1.07	18 1.07	10 0.60	1 0.06
Mean		8.87	17.4	67.37	817	2.53	9.03	15.6 2.15	14.9 2.02	16.6 2.26	10 1.31	8.62 1.14	1 0.14
1	300	10	1	11	504	0.20	2.18	2 0.40	3 0.59	1 0.20	3 0.59	2 0.40	0 0
2	300	8	3	10	677	0.44	1.47	1 0.15	2 0.29	3 0.45	2 0.29	2 0.29	0 0
3	300	10	1	16	857	0.12	1.86	4 0.47	3 0.35	3 0.35	2 0.22	4 0.47	0
4	300	10	0	21	915	0	2.29	5 0.55	3 0.33	4 0.43	6 0.65	3 0.33	0
5	300	6	3	3	207	1.45	1.44	1 0.48	1 0.48	0 0	1 0.48	0 0	0 0
6	300	7	1	8	483	0.21	1.65	1 0.21	2 0.41	2 0.41	1 0.21	2 0.41	0 0
7	300	5	0	4	501	0	0.80	0 0	1 0.2	1 0.2	0 0	1 0.2	1 0.2
8	300	10	0	3	749	0	0.39	1 0.13	1 0.13	1 0.13	0 0	0 0	0 0
Mean		8.25	1.12	9.5	611	0.30	1.51	5 0.30	2 0.35	1.9 0.27	1.9 0.31	1.9 0.26	0.1 0.02

G: Genotype, ID: irradiation dose, OFN: opened fruit number, DEN: diploid embryo number, HEN: haploid embryo number, SN: seed number, DEN per 100 S: diploid embryo number per 100 seeds, HEN per 100 S: haploid embryo number per 100 seeds, TEN: total embryo number per genotype, HEN-HEN per 100 S: haploid embryo number – haploid embryo number per 100 seeds.

Table 4. According to genotype: opened fruit number, diploid embryo number, seed number, diploid embryo number per 100 seeds, haploid embryo number per 100 seeds, and haploid embryo number – haploid embryo number per 100 seeds in terms of embryo type in the second year of the experiment.

G	ID	OFN	DES	HEN	SN	DEN 100 S	HEN 100 S	Embryo type HEN-HEN per 100 seed #					
#	Gy	#	#	#	#	#	#	1	2	3	4	5	6
1	150	10	149	161	949	15.70	16.96	27 2.84	23 2.42	36 3.79	26 2.74	33 3.48	16 1.69
1	200	10	27	45	516	5.23	8.72	10 1.94	12 2.32	15 2.91	5 0.97	2 0.39	1 0.19
1	300	10	1	11	504	0.20	2.18	2 0.40	3 0.59	1 0.20	3 0.59	2 0.40	0 0
Mean		10	59	72.33	656.3	7.04	9.29	13 1.73	12.7 1.78	17.3 2.3	11.3 1.43	12.3 1.42	5.7 0.63
2	150	10	249	373	1702	14.63	21.91	85 4.99	84 4.93	88 5.17	56 3.29	45 2.64	15 0.89
2	200	10	14	79	769	1.82	10.27	25 3.25	28 3.64	15 1.95	7 0.91	4 0.52	0
2	300	8	3	10	677	0.44	1.47	1 0.15	2 0.29	3 0.45	2 0.29	2 0.29	0 0
Mean		9.3	88.7	154	1049	5.63	11.22	37 2.78	38 2.95	35.3 2.52	21.7 1.50	17 1.15	5 0.30
3	150	10	136	309	1949	6.98	15.85	31 1.59	65 3.34	81 4.15	45 2.31	55 2.82	32 1.64
3	200	10	9	50	784	1.15	6.37	9 1.15	8 1.02	12 1.53	11 1.40	8 1.02	2 0.25
3	300	10	1	16	857	0.12	1.86	4 0.47	3 0.35	3 0.35	2 0.22	4 0.47	0
Mean		10	48.7	125	1197	2.75	8.03	14.7 1.07	25.3 1.57	32 2.01	19.3 1.31	22.3 1.44	11.3 0.63
4	150	10	190	176	1007	18.87	17.47	15 1.49	34 3.38	41 4.07	38 3.77	37 3.67	11 1.09
4	200	10	25	111	734	3.40	15.12	16 2.18	17 2.32	30 4.09	23 3.13	23 3.13	2 0.27
4	300	10	0	21	915	0	2.29	5 0.55	3 0.33	4 0.43	6 0.65	3 0.33	0 0
Mean		10	71.7	102	885.3	7.42	11.63	12 1.41	18 2.01	25 2.86	22.3 2.52	21 2.38	4.3 0.45
5	150	10	153	210	1489	10.27	14.10	18 1.21	35 2.35	42 2.82	48 3.22	42 2.82	25 1.68
5	200	9	20	48	931	2.15	5.15	5 0.54	10 1.07	16 1.72	11 1.18	6 0.64	0 0
5	300	6	3	3	207	1.45	1.44	1 0.48	1 0.48	0 0	1 0.48	0 0	0 0
Mean		8.3	58.7	87	875	4.62	6.90	8 0.74	15.3 1.3	19.3 1.51	20 1.63	16 1.15	8.3 0.56

Table 4. (Continued).

G	ID	OFN	DES	HEN	SN	DEN 100 S	HEN 100 S	Embryo type HEN-HEN per 100 seed #					
#	Gy	#	#	#	#	#	#	1	2	3	4	5	6
6	150	10	157	351	1362	11.53	25.77	53 3.89	82 6.02	67 4.92	53 3.89	51 3.74	45 3.31
6	200	8	19	69	546	3.48	12.63	19 3.48	13 2.38	17 3.11	10 1.83	10 1.83	0 0
6	300	7	1	8	483	0.21	1.65	1 0.21	2 0.41	2 0.41	1 0.21	2 0.41	0 0
Mean		8.3	59	142	797	5.07	13.35	24.3 2.53	32.3 2.94	28.6 2.81	21.3 1.98	21 1.99	15 1.10
7	150	10	80	112	898	8.91	12.47	24 2.67	25 2.78	22 2.45	17 1.89	17 1.89	7 0.79
7	200	4	14	52	585	2.39	8.88	20 3.41	14 2.39	10 1.71	0 0	6 1.03	2 0.34
7	300	5	0	4	501	0	0.80	0 0	1 0.2	1 0.2	0 0	1 0.2	1 0.2
Mean		6.3	31.3	56	661.3	3.77	7.38	14.7 2.03	13.3 1.79	11 1.45	5.7 0.63	8 1.04	3.3 0.44
8	150	10	116	318	1277	9.08	24.90	64 5.01	61 4.78	98 7.67	42 3.29	40 3.13	13 1.02
8	200	10	11	85	1675	0.65	5.07	21 1.25	17 1.02	18 1.07	18 1.07	10 0.60	1 0.06
8	300	10	0	3	749	0	0.39	1 0.13	1 0.13	1 0.13	0 0	0 0	0 0
Mean		10	42.3	135.3	1233	3.24	10.1	28.7 2.13	26.3 1.98	39 2.96	20 1.45	16.7 1.24	4.7 0.36

G: Genotype, ID: irradiation dose, OFN: opened fruit number, DEN: diploid embryo number, HEN: haploid embryo number, SN: seed number, DEN per 100 S: diploid embryo number per 100 seeds, HEN per 100 S: haploid embryo number per 100 seeds, TEN: total embryo number per genotype, HEN-HEN per 100 S: haploid embryo number – haploid embryo number per 100 seeds.

4. Discussion

The effects of genotype and gamma ray dose on haploidization in squash were examined in this study. For this purpose 14 squash genotypes and 3 different gamma ray doses (150, 200, and 300 Gy) were used. The results of the study can be summarized as follows. Genotype affected the number of haploid embryos. Embryo number per 100 seeds varied from 12.42 (Genotype 3) to 1.46 embryos per 100 seeds (Genotype 4) in the first year of the experiment. While 13.35 embryos per 100 seeds were obtained from Genotype 6, 6.90 embryos per 100 seeds were observed in Genotype 5 in the second year of the study. Irradiation dose was another important factor in the number of haploid embryos produced. In the first year of the experiment, 9.12 embryos per 100 seeds and 3.53 embryos per 100 seeds were obtained from 150 Gy and 200 Gy, respectively. In the second year, 18.68 embryos per 100 seeds, 9.03 embryos per 100 seeds, and 1.51 embryos per 100 seeds were observed in 150, 200, and 300 Gy irradiation doses, respectively. In both the first and second years of the experiment, the 150 Gy irradiation dose was more successful than the other doses. The irradiation dose also affected the seed number and diploid embryo formation. An increase in the irradiation dose resulted in a decrease in seed number and diploid embryo number. The lowest seed number and diploid embryo formation were observed in 300 Gy irradiation gamma dose. When genotype and irradiation dose are evaluated together, the highest number of haploid embryos was obtained from Genotype 5 and 150 Gy and Genotype 6 and 150 Gy in the first and second year of experiment, respectively.

Kurtar et al. (2002) examined the effects of different genotypes (Eskenderany F1, Acceste F1, Sakız, and Urfa Yerli) and different gamma ray doses (25, 50, 75, 100, 200, 300, and 400 Gy) on haploid embryos obtained through an irradiated pollen technique in squash. They found that 25 Gy and 50 Gy gamma doses were better than other doses, and in terms of genotypes, Eskenderany F1 and Sakız were more successful than other genotypes. Different gamma ray doses (50, 100, 200, and 300 Gy) were tested in another study conducted by Kurtar et al. (2009). They obtained more embryos from the 50 Gy and 100 Gy doses than from 200 Gy and 300 Gy. Berber (2009) used 3 different irradiation doses (50, 100, and 150 Gy) to find a suitable gamma dose and observed the most haploid embryos at 150 Gy. While Kurtar et al. (2002, 2009) found that low irradiation doses performed better than high doses, Berber (2009) found higher doses more successful. Among the tested doses in the current study (150, 200, and 300 Gy), 150 Gy was much better than the other doses. A 150 Gy gamma dose application resulted in almost 3 times more embryos (9.12 embryo per 100 seeds) than 200 Gy (3.53 embryo per 100 seeds) in the first experimental year. In the second year, the 150 Gy gamma dose application gave more embryos (18.68 embryos per 100 seeds) than the 200 Gy (9.03 embryos per 100 seeds) and 300 Gy (9.03 embryos per 100 seeds) irradiation doses.

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References

Baktemur G, Taşkın H, Büyükalaca S (2013). Comparison of different methods for separation of haploid embryo induced through irradiated pollen and their economic analysis in melon (*Cucumis melo* var. *inodorus*). ScientificWorldJournal 2013: 529502.

Başay S, Ellialtıoğlu ŞŞ (2013). Effect of genotypical factors on the effectiveness of anther culture in eggplant (*Solanum melongena* L.). Turk J Biol 37: 499–505.

Berber M (2009). Production of haploids in naked seed pumpkins (*Cucurbita pepo* L. var. *styriaca*) by pollination with irradiated pollen. MSc, Çukurova University, Adana, Turkey.

Chee RP, Leskovar DI, Cantliffe DJ (1992). Optimizing embryogenic callus and embryo growth of a synthetic seed system for sweet potato by varying media nutrient concentrations. J Amer Soc Hortic Sci 117: 663–667.

Chun L, Jin-Feng C, Chun-Tao Q, Xiao-Qing Z, Yong-Bing Z (2006). Studies on induction of haploid cucumbers by irradiated pollen pollination and their characterization. Sci Agric Sinica 39: 1428–1436.

Claveria E, Garcia-Mas J, Dolcet-Sanjuan R (2005). Optimization of cucumber doubled haploid line production using in vitro rescue of in vivo induced parthenogenic embryos. J Amer Soc Hortic Sci 130: 555–560.

Cuny F, Grotte M, Dumas de Vaulx R, Rieu A (1993). Effects of gamma irradiation of pollen on parthenogenetic haploid production in muskmelon (*Cucumis melo* L.). Environ Exp Bot 33: 301–312.

Faris NM, Nikolova V, Niemirowicz-Szczytt K (1999). The effect of gamma irradiation dose on cucumber (*Cucumis sativus* L.) haploid embryo production. Acta Physiol Plant 21: 391–396.

Gémesné JA, Venczel G (1996). *In vitro* gynogenesis induction in zucchini (*Cucurbita pepo* L. convar. *giromontiina* Duch) lines. In: Proceedings of the 6th Eucarpia Meeting on Cucurbit Genetics and Breeding, pp. 200–201.

Godbole MG, Murthy HN (2012a). Parthenogenetic haploid plants using gamma irradiated pollen in snapmelon (*Cucumis melo* var. *momordica*). Plant Cell Tiss Org 109: 167–170.

Godbole MG, Murthy HN (2012b). *In vitro* production of haploids via parthenogenesis in culinary melon (*Cucumis melo* var. *acidulous*). Indian J Biotechnol 11: 495–497.

Gursoz N, Abak K, Pitrat M, Dumas de Vaulx R (1991). Obtention of haploid plants induced by irradiated pollen in watermelon (*Citrullus lanatus*). Cucurbit Genetics Cooperative Report 14: 109–110.

Kurtar ES, Balkaya A, Ozbakir M, Ofluoglu T (2009). Induction of haploid embryo and plant regeneration via irradiated pollen technique in pumpkin (*Cucurbita moschata* Duchesne ex Poir). Afr J Biotechnol 8: 5944–5951.

Kurtar ES, Sarı N, Abak K (2002). Obtention of haploid embryos and plants through irradiated pollen technique in squash (*Cucurbita pepo* L.). Euphytica 127: 335–344.

Metwally EI, Moustafa SA, El-Sawy BI, Harounl SA, Shalaby TA (1998a). Production of haploid plants from *in vitro* culture of unpollinated ovules of *Cucurbita pepo.* Plant Cell Tiss Org 52: 117–121.

Metwally EI, Moustafa SA, El-Sawy BI, Shalaby TA (1998b). Haploid plantlets derived by anther culture of *Cucurbita pepo.* Plant Cell Tiss Org 52: 171–176.

Mohamed MF, Refaei EFS (2004). Enhanced haploids regeneration in anther culture of summer squash (*Cucurbita pepo* L.). Cucur Genet Coop Rep 27: 57–60.

Nikolova V, Alexandrova M (2001). Gynogenesis in a dihaploid line of cucumber (*Cucumis sativus* L.). Cucur Genet Coop Rep 24: 20–21.

Olszewska D, Kisiala A, Niklas-Nowak A, Nowaczyk P (2014). Study of in vitro anther culture in selected genotypes of *Capsicum* genus. Turk J Biol 38: 118–124.

Sandı B (1998). Kabuksuz çerezlik kabakta (*Cucurbita pepo* L.) haploid bitki elde etme olanakları. MSc, Ankara University, Ankara, Turkey (in Turkish).

Sarı N, Abak K, Pitrat M, Rode JC, Dumas de Vaulx R (1994). Induction of parthenogenetic haploid embryos after pollination by irradiated pollen in watermelon. HortScience 29: 1189–1190.

Taşkın H, Yücel NK, Baktemur G, Çömlekçioğlu S, Büyükalaca S (2013). Effects of different genotypes and gamma ray doses on haploidization with irradiated pollen technique in watermelon (*Citrullus lanatus* L.). Can J Plant Sci 93: 1165-1168.