Yüzüncü Yıl Üniversitesi
Tarım Bilimleri Dergisi
(YYU Journal of Agricultural Science)

http://dergipark.gov.tr/yyutbd

Araştırma Makalesi (Research Article)

Dihaploidization in Promising Summer Squash Genotypes (*Cucurbita pepo* L.) via Irradiated Pollen Technique

Ertan Sait KURTAR[*1], Musa SEYMEN[2], Ayşe Nur ÇETİN[3], Önder TÜRKMEN[4]

[1,2,4] Selcuk University, Horticulture Department of Agriculture Faculty, 42010, Konya, Turkey
[3]Ahi Evran University, Horticulture Department of Agriculture Faculty, Kırşehir, Turkey
[1]https://orcid.org/0000-0002-7203-7430 [2]https://orcid.org/0000-0002-2742-137X [3]https://orcid.org/0000-0002-0826-1243
[4]https://orcid.org/0000-0003-3218-6551
*Corresponding author e-mail: ertansaitkurtar@selcuk.edu.tr

Article Info

Received: 26.09.2020
Accepted: 01.12.2020
Online Published 30.03.2021
DOI: 10.29133/yyutbd.800475

Keywords

Cucurbitaceae,
Haploid,
Plant tissue culture,
Pure lines.

Abstract: Summer squash (*Cucurbita pepo* L.) is one of the important vegetable species preferred by the people of Turkey in terms of their nutritional value. F1 hybrid summer squash varieties are widely used both in the open field and protected cultivation. The majority of these varieties come from abroad, and our dependence on abroad continues. In this study, the effectiveness of the dihaploidization method, which has an important place in variety breeding studies, was investigated to reduce our import dependency and to produce new F1 hybrid varieties that are high yielded, quality, resistant to diseases and pests. In this context, 14 summer squash genotypes were used as plant material. Dihaploidization studies were carried out using the irradiated pollen technique. In total, 165 flowers were pollinated and 64 fruits, 7034 seeds, 521 embryos, and 144 plants were obtained. 111 pants were successfully acclimatized and cultivated in controlled conditions. As a result of ploidy analysis, 28 plants were haploid (2n=x=20), 77 were diploid (2n=2x=40) and 6 were mixoploid (containing diploid and haploid cells). Haploid plants were doubled with 1% 42olchicines treatment, grown in the greenhouse, selfed, and seeds were obtained.

Nitelikli Yazlık Kabak (*Cucurbita pepo* L.) Genotiplerinde Işınlanmış Polen Tekniği ile Dihaploidizasyon

Makale Bilgileri

Geliş: 26.09.2020
Kabul: 01.12.2020
Online Yayınlanma 30.03.2021
DOI: 10.29133/yyutbd.800475

Anahtar kelimeler

Cucurbitaceae,
Haploid,
Bitki doku kültürü,
Saf hatlar.

Öz: Yazlık kabak (*Cucurbita pepo* L.) sahip olduğu besleyici değer ile ülkemizde insanlar tarafından tercih edilen önemli bir sebze türüdür. F1 hibrid yazlık kabak çeşitleri hem açık tarla hem de örtüaltı yetiştiriciliğinde yaygın olarak kullanılmakta, bu çeşitlerin büyük çoğunluğu yurt dışı kaynaklıdır ve yurt dışı bağımlılığımız devam etmektedir. Çalışmada, yüksek verimli ve kaliteli, hastalık ve zararlılara dayanıklı yeni F1 hibrit yazlık kabak çeşitlerini üretmek, yurt dışı bağımlılığımızı azaltmak amacıyla, çeşit ıslahı çalışmalarında önemli bir yere sahip olan dihaploidizasyon metodunun etkinliği araştırılmıştır. Bu bağlamda, 14 yazlık kabak genotipi bitkisel materyal olarak kullanılmış, dihaploidizasyon çalışmaları ışınlanmış polen tekniği kullanılarak gerçekleştirilmiştir. Toplamda 165 çiçek tozlanmış, 64 meyve, 7034 tohum, 521 embriyo ve 144 bitki elde edilmiştir. 111 bitki başarılı bir şekilde dış koşullara alıştırılmış ve kontrollü koşullarda yetiştirilmiştir. Ploidi analizleri sonucunda, 28 bitki haploid (n = 20), 77 bitki diploid (2n = 40) ve 6 bitki miksoploid (haploid ve diploid hücreli) yapıda olmuştur. Haploid bitkiler %1'lik kolhisin uygulamasıyla katlanmış, sera koşullarında yetiştirilmiş, kendilenmiş ve tohumları alınmıştır.

1. Introduction

Turkey allows for the cultivation of many vegetable species related to the *Cucurbitaceae* family with its favorable climatic conditions and our total summer squash production is 474.527 tons (TÜİK, 2018). *Cucurbita pepo* L., defined as summer squash, has a very wide range in our country's food culture. Although squash is generally produced for fruit, its seeds are also used as appetizers, high-quality edible oil, and food production materials, which are demanded in the market, thus it is cultivated in both open field and greenhouse conditions. Some squash populations with mostly domestic names exist in many regions in Turkey. However, these local varieties are preferred their taste and flavor, they are not at the desired agricultural traits as high-yield, quality, and especially resistant to disease and pests. Thus, superior F_1 hybrid summer squash varieties are widely used in vegetable production. As in many vegetable varieties, F1 hybrid summer squash varieties are mostly imported and our foreign dependency continues. Reducing foreign dependency on seed production is among the priority subject in Turkey. In this context, the use of the dihaploidization technique will provide a great convenience in breeding efforts to reduce our foreign dependency in summer squash, to reveal our domestic varieties that are high yielded, quality, resistant to diseases and pests, and to obtain new F1 hybrid varieties.

The dihaploidization technique is one of the plant tissue culture techniques and they are widely used in F1 hybrid seed production. The most important advantage of this technique shortens the cultivar breeding process. It also increases breeding efficiency by reducing the size of the population that needs to be addressed to achieve the targeted genotype. In classical breeding, the fact that selection cannot be done effectively in early generations is an important problem. Achieving double-haploid plants in a short time with the dihaploidization technique enables an effective selection in early generations and accelerates responding to changing consumer demands (Forster and Thomas, 2005).

Classical breeding processes take a long time because the flower types of the species that belong to the *Cucurbitaceae* family have very different characteristics (monoic, andromonoic, gynoic, gynomonoic, hermaphrodite) and naturally high rate of open-pollinated. However, the haploid plant obtaining technique, which has been used successfully in some species instead of classical breeding, has important advantages. The purpose of dihaploidization (DH) technique is to obtain male (anther - pollen culture), female (ovule - ovary culture), and/or parthenogenesis (irradiated pollen technique) originating haploid (n) plants and to generate 100% pure (homozygous) lines (2n) by doubling the chromosome numbers of haploid plants with various antimitotic agents (colchicine, oryzalin, trifluralin, amiprofos methyl). Thus, the purification process, which takes many years (8-10 years) especially in open-pollinated species, can be carried out in a shorter time (1-2 years). The breeding of hybrid cultivar can be completed in a short time with the desired agronomic traits and high combination ability from these pure lines obtained.

Considering the species of the *Cucurbitaceae* family, irradiated pollen is the most used technique in dihaploidization process. In this technique, the pollen is irradiated by different irradiation source (usually with Co^{60}-induced gamma-ray), but since the germination capabilities continue, it stimulates the female organ and causes the parthenogenetic haploid embryos. With the use of this technique, successful results have been obtained in watermelon (Gürsöz et al., 1991; Sarı et al., 1994), melon (Sauton and Dumas de Vaulx, 1987; Sarı et al., 1992; Abak et al., 1996; Lotfi et al., 2003), cucumber (Truong-Andre, 1988; Sauton, 1989; Çağlar and Abak, 1999; Dolcet-Sanjuan et al., 2006), summer squash (Kurtar, 1999; Kurtar et al., 2002; Berber, 2009; Bektemur et al., 2014; Kurtar et al., 2017), pumpkin (Kurtar et al., 2009) and winter squash (Kurtar and Balkaya, 2010) species. The most important factor that restricts the effectiveness of the irradiated pollen technique is genotype dependence. Besides, some factors such as irradiation and pollination studies, the composition of the media, culture conditions, embryo development stages, and growing season and conditions of donors can also affect the haploidy frequency.

In this study, it is aimed to determine the effectiveness of the dihaploidization process on producing pure lines in summer squash selected from our gene pool via irradiated pollen technique.

2. Materials and Methods

The research was carried out in the laboratory and greenhouse of Selcuk University Faculty of Agriculture, Department of Horticulture, Vegetable Growing and Breeding in 2018 and 2019. Seeds of 14 genotypes obtained from the gene pool of this department were sown in trays filled with peat-moss on May 4, 2018, and seedlings at the 2-3-leaf stage were planted in the greenhouse at a distance of 1.2 x 1 m on 21 May 2018. Twenty plants were used for each genotype (Figure 1). In the growing period, considering the soil analysis results, 8 kg N (Nitrogen), 6 kg P_2O_5 (Phosphorus), and 7 kg K_2O (Potassium) were supplied per decare with drip irrigation, and pest and disease management were applied regularly.

Figure 1. Planted seedlings in the greenhouse and growing squash plants.

Anthers were collected one day before the anthesis from genotypes when the plants were in the full-flowering period and the female flowers to be pollinated the next day were isolated with cloth bags. The anthers were irradiated on the same day with a dose of 150 Gy gamma rays in TAEK (Turkish Atomic Energy Authority) (Figure 2). The isolated female flowers were pollinated with irradiated pollen the next morning and re-isolated to prevent undesired pollen contamination. After 2-3 days of pollination, cloth bags were removed and the fruits were labeled.

Figure 2. Female flower isolated 1 day before anthesis, anthers collected 1 day before anthesis, and irradiation of anthers on Co^{60} equipment.

About 3-4 weeks after pollination, the fruits were harvested before they were not fully ripened and the embryos were not aged and lost their regeneration ability. Harvested fruits were washed under tap water before extraction, cleaned from external contamination, weighed on a digital scale with a sensitivity of 2 g after drying. Then the fruit stems were removed and fruits were soaked in a 20% commercial bleach solution for 30 min. Finally, after thoroughly pulverizing with 96% ethyl alcohol under a sterile bench, surface sterilization was performed by burning. The surface-sterilized fruits were carefully cut with the help of a sterile knife without damaging the seeds, and all the seeds (except very small, dice or scar) were opened one by one and the embryos were cultured.

All the embryos obtained from the opened seeds, less than half the seed size were taken into culture tubes containing approximately 10 ml of modified E20A medium. Because, according to our previous experiences, embryos larger than half the seed size have formed completely diploid plants. During this period, embryos were kept in the climate room, whose temperature was 28 ± 1 °C, the photoperiod was 16/8 day-night, and the illumination was set to 5000 lux with daylight type fluorescent lamps (Kurtar and Balkaya, 2010) (Figure 3). The composition of the modified E20A medium is presented in Table 1.

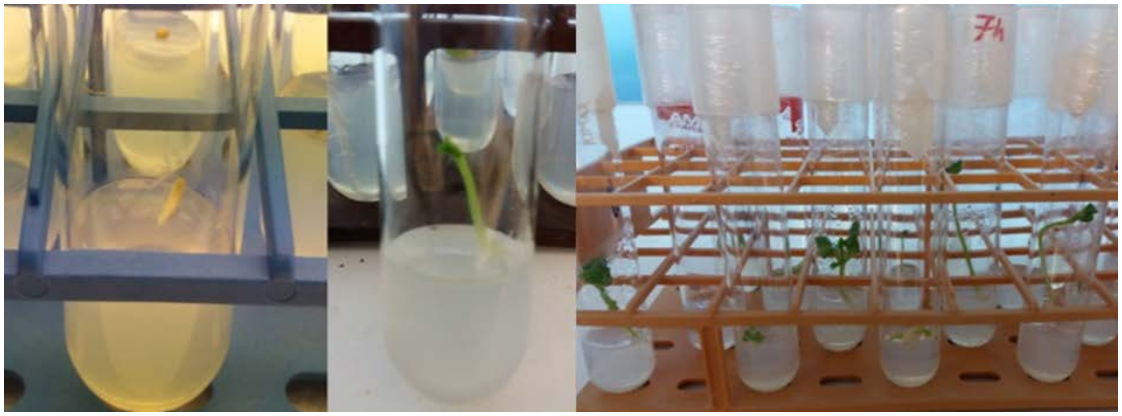

Figure 3. Cultured and developed embryos.

Table 1. The composition of modified E20A medium

Macroelements (mg l^{-1})		Microelements (mg l^{-1})	
KNO_3	1075.0	$MnSO_4.H_2O$	11.065
NH_4NO_3	619.0	$ZnSO_4.7H_2O$	1.812
$MgSO_4.7H_2O$	206.0	H_3BO_3	1.575
$CaCl_2.2H_2O$	156.5	KI	0.345
KH_2PO_4	71.0	$Na_2MoO_4.2H_2O$	0.094
$Ca(NO_3)_2.4H_2O$	25.0	$CuSO_4.5H_2O$	0.008
$NaH_2PO_4.4H_2O$	19.0	$CoCl_2.6H_2O$	0.008
$(NH_4)_2SO_4$	17.0		
KCl	3.5		
Na_2EDTA : 37.3 mg l^{-1} , $FeSO_4.7H_2O$: 27.8 mg l^{-1}			
Vitamins and amino acids		Others	
Myo-inositol	50.300	Sucrose	20.00 g l^{-1}
Pyridoxine-HCl	5.500	Agar-Agar	8.00 g l^{-1}
Nicotinik asit	0.700	IAA	0.01 mg l^{-1}
Thiamine-HCl	0.600	pH	5.90
C. Pantothenate	0.500		
Glycine	0.100	Autoclave: 1.1atm, 121 °C, 15 min	
Biotin	0.005		

To prevent the loss and increase the number of plants, micro-propagation was carried out in developed plants (had a favorable root and shoot ratio) under laboratory conditions for 2-3 times. Micro-propagation was realized in MS medium (Murashige and Skoog, 1962) with the addition of 0.1 mg l^{-1} IAA and 1.0 mg l^{-1} BAP (Figure 4).

The plantlets were acclimatized to the open field conditions as reported by Kurtar and Balkaya (2010). The ploidy levels of the plants were determined by both stomatal (stoma number and stoma size) and morphological observations. As a result of ploidy analyses, haploid plants were doubled by applying 1% colchicine solution to the shoot-tips of the plants (Figure 5). Double-haploid plants were grown in the climate room and 69 plants (2-3 plants from each dihaploid line) were planted in a heated greenhouse in April 2019 (Figure 6).

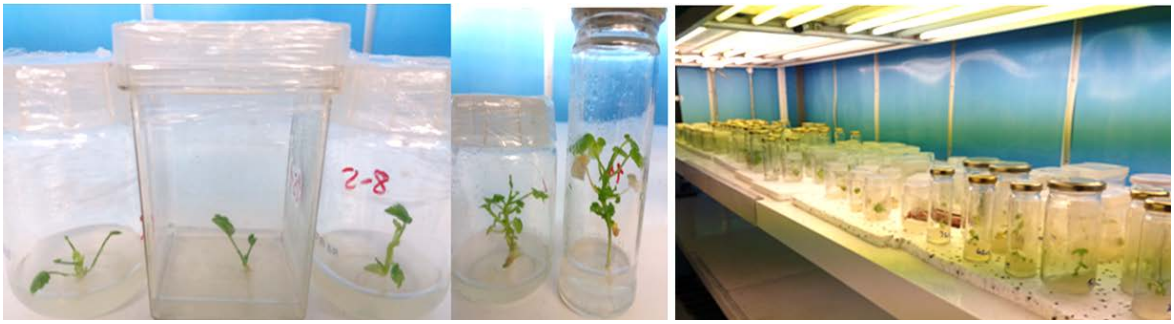

Figure 4. Micro-propagated plants.

Figure 5. Chromosome doubling in haploid plants.

Figure 6. Double-haploid plants grown in the climate room and planted in the greenhouse.

2.1. Data analyses

Since the number of fruits of the genotypes is not equal and a different number of materials are used, only the averages of the data are provided.

3. Results and Discussion

3.1. Effects of pollination with irradiated pollen on fruit weight, seed formation, and embryo stimulation

In pollination studies, the number of pollinated flowers (PF), the number of fruit set (FS), the fruit set rate (FR), and the average fruit weight (AW) were evaluated and presented in Table 2. As a result of the pollination studies, 165 flowers were pollinated, 64 fruits were obtained from the

pollinated flowers and the fruit set rate was 38.8%. Fruit set ratio varied between 20% (G6) and 61.5% (G7) and the average fruit weight (AW) ranged from 1650 g (G6) to 4465 g (G4).

Table 2. The number of pollinated flowers (PF), the number of fruit set (FS), the fruit set rate (FR, %), and the average fruit weight (AW, g) in genotypes (G).

G	PF	FS	FR	AW
1	14	5	35.7	1280
G2	17	7	41.2	1875
G3	9	3	33.3	3480
G4	8	2	25.0	4465
G5	13	6	46.2	1785
G6	5	1	20.0	1650
G7	13	8	61.5	2870
G8	9	4	44.4	1965
G9	15	6	40.0	2845
G10	13	5	38.5	3565
G11	11	4	36.4	4060
G12	8	3	37.5	1895
G13	14	4	28.6	3085
G14	16	6	37.5	3995
	165	64	38.8	2773

As a result of the extraction of 64 fruits, 7034 seeds and 521 embryos were obtained. The average number of seeds per fruit was 109.9 and the number of embryos per fruit was 8.14. Of the 521 embryos obtained, 144 transformed into a complete plant and the conversion rate to the plant was 27.6%. Among the genotypes, the average number of seeds ranged from 69 (G8) to 183 (G9), the number of embryos per fruit was between 2.2 (G14) and 14.7 (G3), and the conversion rate to the plant was between 19.0% (G2) and 38.2% (G12) (Table 3). These results are following our previous studies in the summer squash (Kurtar, 1999; Kurtar et al., 2002; Kurtar et al., 2017).

Table 3. The number of fruit (FS), the number of seeds (SN), the average number of seeds (AS), the total number of embryos (NE), the number of embryos per fruit (ENF), the number of embryos transformed into plants (ENP) and the rate of conversion to plants (RP, %) in genotypes (G).

G	FS	SN	AS	NE	ENF	ENP	RP
G1	5	490	98	68	13.6	13	19.1
G2	7	819	117	84	12.0	16	19.0
G3	3	489	163	44	14.7	11	25.0
G4	2	148	74	9	4.5	3	33.3
G5	6	654	109	23	3.8	6	26.1
G6	1	107	107	11	11.0	3	27.3
G7	8	904	113	62	7.8	17	27.4
G8	4	276	69	24	6.0	8	33.3
G9	6	1098	183	51	8.5	18	35.3
G10	5	405	81	42	8.4	16	38.1
G11	4	288	72	29	7.3	9	31.0
G12	3	342	114	34	11.3	13	38.2
G13	4	488	122	27	6.8	7	25.9
G14	6	528	88	13	2.2	4	30.8
	64	7034	109.9	521	8.14	144	27.6

3.2. Ploidy analyses and dihaploidization process

The ploidy levels of the plants were determined by both stomatal (stoma number, stoma size) and morphological observations. While the average stoma length and stoma widths were 32.24 and 21.67 µm in diploids, these values were determined as 20.41 and 16.84 µm in haploids. While the number of stomata per unit area (mm^2) was 306.2 in diploids, it was 435.1 in haploids.

While diploid plants formed larger leaves and habitus, haploid plants had smaller leaves and plants. Besides, pollen formation was not observed in the anthers of haploid plants (Figure 7). Similar results have been found in the previous studies in squash (Kurtar, 1999; Kurtar et al., 2002; Kurtar et al., 2017), pumpkin (Kurtar et al., 2009), and winter squash (Kurtar and Balkaya, 2010).

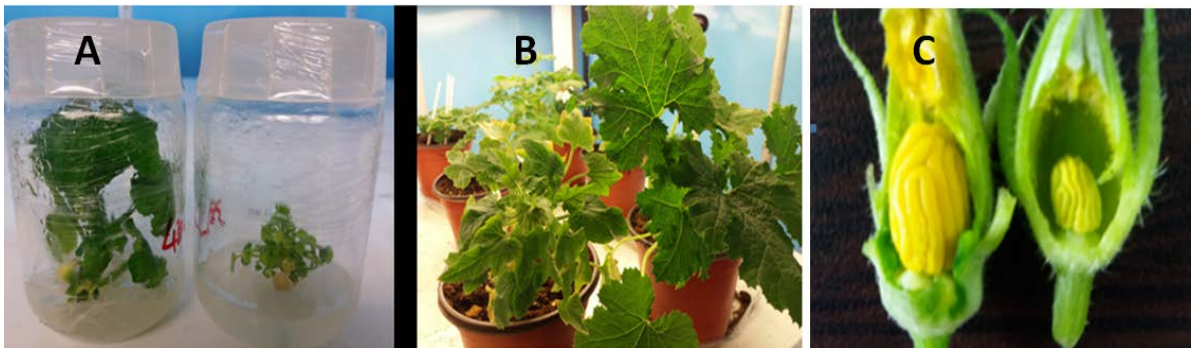

Figure 7. Diploid (left) and haploid (right) plantlets developing *in vitro* conditions (A); Haploid (left) and diploid (right) plants acclimatized to open field conditions (B); Anthers of diploid (left - fertile) and haploid (right - sterile) plants (C).

As a result of the ploidy analyses of 111 plants, 28 plants were haploid (n = x = 20), 77 plants were diploid (2n = 2x = 40) and 6 plants were mixoploid (containing diploid and haploid cells) (Table 4).

Table 4. The number of plants (PS), the number of acclimatized and examined plants (AP), haploid plants (H), diploid plants (D), mixoploid plants (M) and haploidy efficiency (HE, %) in genotypes (G).

G	PS	AP	H	D	M	HE
G1	13	11	2	9	-	18.2
G2	16	13	4	8	1	30.8
G3	11	9	3	6	-	33.3
G4	3	2	-	2	-	0.0
G5	6	4	1	3	-	25.0
G6	3	1	-	1	-	0.0
G7	17	14	4	8	2	28.6
G8	8	5	2	3	-	40.0
G9	18	12	3	7	2	25.0
G10	16	14	3	11	-	21.4
G11	9	8	2	6	-	25.0
G12	13	11	3	7	1	27.3
G13	7	5	1	4	-	20.0
G14	4	2	-	2	-	0.0
	144	111	28	77	6	25.2

Haploid production efficiency (HE) has also been determined by considering the criteria of 100 seed/haploid plants, 100 embryo/haploid plants, and haploid plants per fruit in genotypes. As a result of pollination with irradiated pollen, haploid plants could not be obtained from some genotypes (G4, G6, and G14), while the number of haploid plants per fruit varied between 0.25 (G13) and 1.00 (G3 and G12). The number of haploid plants in 100 seeds and the number of haploid plants in 100 embryos ranged from 0.15 (G5) to 0.88 (G12) and 2.90 (G1) and 8.82 (G12), respectively (Table 5). These findings in accordance with our previous studies in summer squash (Kurtar, 1999; Kurtar et al., 2002; Kurtar et al., 2017), pumpkin (Kurtar et al., 2009), and winter squash (Kurtar and Balkaya, 2010).

Table 5. The number of haploid plants (HP), the number of fruits (NF), the number of seeds (NS), the total number of embryos (TE), the number of haploid plants per fruit (HPF), the number of haploid plants in 100 seeds (HPS), the number of haploid plants in 100 embryos (HPE) in genotypes (G).

G	HP	NF	NS	TE	HPF	HPS	HPE
G1	2	5	490	68	0.40	0.41	2.90
G2	4	7	819	84	0.57	0.49	4.76
G3	3	3	489	44	1.00	0.61	6.81
G4	-	2	148	9	0.00	0.00	0.00
G5	1	6	654	23	0.17	0.15	4.34
G6	-	1	107	11	0.00	0.00	0.00
G7	4	8	904	62	0.50	0.44	6.45
G8	2	4	276	24	0.50	0.72	8.33
G9	3	6	1098	51	0.50	0.27	5.88
G10	3	5	405	42	0.60	0.74	7.14
G11	2	4	288	29	0.50	0.69	6.89
G12	3	3	342	34	1.00	0.88	8.82
G13	1	4	488	27	0.25	0.20	3.70
G14	-	6	528	13	0.00	0.00	0.00
	28	64	7034	521	0.44	0.40	5.37

The double-haploid plants have been shown to very different growth characteristics and it has been demonstrated that the dihaploidization technique is also important for creating variation beyond obtaining pure lines (Figure 8).

Figure 8. The appearance of double-haploid plants with different formations in the greenhouse.

4. Conclusion and perspective

The dihaploidization technique, which enables obtention of pure homozygous lines in many vegetable types and greatly shortens the breeding time, improves the breeding efficiency, and is successfully applied in the summer squash. However, the most important factor preventing the widespread use of the technique is that it is genotype-dependent and the haploid frequency shows a large variation among the genotypes.

It has been observed that the dihaploid lines we have obtained have very different growth characteristics and that the dihaploidization technique is an important technique for creating variation besides pure line production. In the light of our results, we believe that it will increase the success of working with genotypes with high haploid frequency, hybridization between high frequency and low-frequency genotypes, the spread of irradiation and pollination studies to a wider vegetation period in future studies. Also, as an alternative to the irradiated pollen technique, anther-microspore or ovule-ovary cultures should be engaged (Kurtar et al., 2020).

In this context, our goals are to continue irradiation and pollination activities with high haploid frequency genotypes, on the other hand, to produce suitable candidates for the breeding of F_1 hybrid summer squash with promising dihaploid lines that have desired agronomic traits we obtained as a result of the study.

Acknowledgments

This research was funded by the Scientific Research Projects Coordination Unit of Selcuk University, Turkey (Project No.18401059).

References

Abak, K., Sarı, N., Paksoy, M., Yılmaz, H., Aktaş¸ H., & Tunalı, C. (1996). Genotype response to haploid embryo induction with pollination by irradiated pollen in melon, obtaining of dihaploid lines, determination of haploid plants by different techniques. *Turkish Journal of Agriculture and Forestry, 20*, 425–430.

Bektemur, G., Yücel, N. K., Taşkın, H., Çömlekçioğlu, S., & Büyükalaca, S. (2014). Effects of different genotypes and gamma-ray doses on haploidization using irradiated pollen technique in squash. *Turkish Journal of Biology*, 38, 318–327.

Berber, M. (2009). *Production of haploids in naked seed pumpkins (Cucurbita pepo L. var. styriaca) by pollination with irradiated pollen.* Ins of Nat and App Sci Univ of Çukurova, MSc Thesis, 144 p.

Çağlar, G., & Abak, K. (1999). Obtention of *in vitro* haploid plants from *in situ* induced haploid embryos in cucumber (*Cucumis sativus* L.). *Turkish Journal of Agriculture and Forestry, 23*, 283–290.

Dolcet-Sanjuan, R., Claveria, E., & Garcia-Mas, J. (2006). Cucumber (*Cucumis sativus* L.) dihaploid line production using *in vitro* rescue of *in vivo* induced parthenogenic embryos. *Acta Horticulturae, 725*, 837–844.

Forster, B. P., & Thomas, W. T. B. (2005). Doubled haploids in genetics and plant breeding. *In: Janick J(ed) Plant Breeding Review, 25*, 57–88.

Gürsöz, N., Abak, K., Pitrat, M., Rode, J. C., & Dumas de Vaulx, R. (1991). Obtention of haploid plants induced by irradiated pollen in watermelon (*Citrullus lanatus*). *Cucurbit Genetic Cooperative, 14*, 109–110.

Kurtar, E. S. (1999). *Research on the effects of genotypes and growing seasons on in situ haploid embryo induction and in vitro plant obtention via irradiated pollen in squash.* PhD Thesis, Univ of Cukurova, 203 p.

Kurtar, E. S., & Balkaya, A. (2010). Production of *in vitro* haploid plants from *in situ* induced haploid embryos in winter squash (*Cucurbita maxima* Duchesne ex Lam.) via irradiated Pollen. *Plant Cell Tiss Org Cult, 102*, 267–277.

Kurtar, E. S., Balkaya, A., Göçmen, M., & Karaağaç, O. (2017). Dihaploidization in squash genotypes (*Cucurbita spp*) as a rootstock candidate for cucumber (*Cucumis sativus* L.) via irradiated pollen technique. *Selcuk Journal of Agriculture and Food Science, 31(1),* 34-41.

Kurtar, E. S., Balkaya, A., Özbakır, M., & Ofluoglu, T. (2009). Induction of haploid embryo and plant regeneration via irradiated pollen technique in pumpkin (*Cucurbita moschata* Duchesne ex. Poir). *African Journal of Biotechnology, 8,* 5944–5951.

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

Kurtar, E.S., Seymen, M., & Kal, Ü. (2020). An overview of doubled haploid plant production in *Cucurbita* species. *Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi, 30(3),* 510-520.

Lotfi, M., Alan, A. R., Henning, M. J., Jahn, M. M., & Earle, E. D. (2003). Production of haploid and doubled haploid plants of melon (*Cucumis melo* L.) for use in breeding for multiple virus resistance. *Plant Cell Reports, 21,* 1121–1128.

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiology Plantarum, 15,* 473-497.

Sarı, N., Abak, K., & Pitrat, M. (1994). Induction of parthenogenetic haploid embryos after pollination by irradiated pollen in watermelon, *Hort Science, 29 (10),* 1189-1190.

Sarı, N., Abak, K., Pitrat, M., & Dumas de Vaulx, R. (1992). Induction of parthenogenetic haploid embryos and plant obtention in melon (*Cucumis melo* L. var. inodorus Naud and *C. melo* L. var. reticulatus Naud). *Turkish Journal of Agriculture and Forestry, 16,* 302-314.

Sauton, A. (1989). Haploid gynogenesis in *Cucumis sativus* induced by irradiated pollen. *Cucurbit Genetic Cooperative, 12,* 22–23.

Sauton, A., & Dumas De Vaulx, R. (1987). Obtention de plantes haploides chez le melon (*Cucumis melo* L.) par gynoge´ne`se induite par du pollen irradie´. *Agronomie, 7,*141–148.

Truong-Andre, I. (1988). *In vitro* haploid plants derived from pollinisation by irradiated pollen on cucumber. *In: Proc of the Eucarpia Meet on cucurbit genetics and breeding.* May 31– June 2, Avignon-Montfavet, pp 143–144.

TÜİK, (2018). Turkish Statistical Institute. Retrieved on April 7, 2018, from http://www.tuik.gov.tr