Selcuk Journal of Agriculture and Food Sciences

https://dergipark.org.tr/tr/pub/selcukjafsci

Research Article

(2024) 38(3), 571-578 DOI:10.15316/SJAFS.2024.051 e-ISSN: 2458-8377 Selcuk J Agr Food Sci

Obtaining Haploid Plants Via Anther Culture in Some Eggplant Varieties

Namık Kemal Yücel[1,*]

[1]Selçuk University, Faculty of Agriculture, Department of Horticulture, Konya, Türkiye

HIGHLIGHTS

- Modified callus culture was detected in eggplant
- Regeneration medium varies depending on the species.
- Determination of ploidy of plants obtained from anther culture is important.

Abstract

In plant breeding studies, anther culture is used to breed hybrid varieties, to transform tetraplaid plants that can be obtained through interspecific somatic hybridization into fertile dihaploid plants, and in recent years, to obtain diploid in protoplast fusion of haploid embryogenic calli obtained from anther culture. The frequency of obtaining haploid plants in anther culture depends on many factors such as the variety, the season in which it was taken and the use of suitable media for regeneration. It was aimed to determine the most suitable regeneration medium for anther culture in Aydın Black and Kemer eggplant varieties, which are widely grown in our country. In the anther culture study, C (callus), R (regeneration) and V3 media recommended by Dumas de Valux et al. (1982) were used as nutrient media. However, as C (callus) medium, Dumas de Vaulx et al. (1982) 3 different doses of 2,4 D, Kinetin and sucrose of the C medium reported were tested. The highest plant yield (36.4%) of the Aydin Siyahı variety was obtained from the modified Dumas de Vaulx et al. (1982), from 1 mg/l 2.4-D +1 mg/l Kinetin + 120 g/l sucrose medium, the highest plant yield (33.8%) of the Kemer variety was reported by Dumas de Vaulx et al. (1982), obtained from 5 mg/l 2.4-D + 5 mg/l Kinetin + 120 g/l sucrose medium. Of the 79 plants obtained from anther culture and whose ploidy level was examined, 60 were determined as haploid, 13 as diploid, 4 as triploid, 1 as tetraploid and 1 as mixoploid.

Keywords: *Solanum melongena* , anther culture, haphoid plant, callus medium

1. Introduction

Eggplant is the third most important vegetable in terms of production within the Solanaceae family, after potatoes and tomatoes, and is also very valuable in terms of its vitamin and mineral content. Eggplant constitutes approximately 5% (59 million tons) of the world's total vegetable production (1173 million tons). According to FAO 2022 data, 93% of eggplant production is produced in Asia and 6% in Africa, Europe and America (FAO 2024). According to Gebeloğlu and Ellialtıoğlu (2022), there are 3 cultured species (*Solanum melongena, Solanum aethiopicum* and *Solanum macrocarpon*). Anther culture; It is the name given to the phenomenon of isolating anthers containing immature microspores from the buds and placing them in

Citation: Yücel NK, (2024). Obtaining Haploid Plants Via Anther Culture in Some Eggplant Varieties. *Selcuk Journal of Agriculture and Food Sciences*, 38(3), 571-578. https://doi.org/10.15316/SJAFS.2024.051

Correspondence: nkyucel@selcuk.edu.tr

Received date: 12/11/2024

Accepted date: 11/12/2024

artificial nutrient media under *in vitro* conditions, where haploid embryos are obtained from the microspores (Ellialtıoğlu et al. 2012). Anther culture is a technique used in eggplant since the 1980s, and the haploid plants obtained are used in F1 hybrid breeding and mutation breeding. (Alpsoy and Şeniz, 2007; Seguí-Simarro, 2016; Rotino, 2016). Obtaining homozygous plants (DH) used in F1 variety breeding from haploid plants obtained from anther culture shortens the breeding period. However, the production of haploid plants varies depending on the species, the period in which microspores are collected, and the physical and chemical structure of the regeneration environment (Calabuig-Serna et al. 2020; Salas et al. 2012). The anther culture technique has been used in eggplant breeding for more than 40 years and very few successful results have been obtained. (Khatun et al. 2006; Kumar et al. 2003; Alpsoy and Şeniz 2007; Salas et al. 2011; and Başay and Ellialtıoğlu 2013).

In eggplant anther culture studies, it has been determined that the most suitable bud harvesting time for *Solanum melongena* is at the last stage of the single-nucleated microspore, that is, morphologically, the petal appears in a bitter yellow-yellow color and 2 mm. (Vural et al. 2019; Salas et al. 2012; Mir et al. 2021).

The aim of this study is to analyze Kemer and Aydın Siyahı varieties by Dumas de Vaulx et al. (1982) reported that the C medium was modified and 3 different doses of 2,4 D, Kinetin and sucrose were tested to increase the formation of embrogenic plants. The ploidy levels of the obtained plants were determined by flow-cytometry method.

2. Materials and Methods

Research; It was carried out in Çukurova University Faculty of Agriculture Department of Horticulture Tissue Culture Laboratory and Research and Application Field, Çukurova University Biotechnology Center and Batı Akdeniz Agricultural Research Institute Directorate (BATEM) Vegetable and Ornamental Plants Department Application Field and Laboratory.

2.1. Plant material

The seeds of the plant materials used in the study were obtained from the Batı Akdeniz Agricultural Research Institute (BATEM). Seeds *of Solanum melongena* cv Kemer and Aydın Siyahı eggplant varieties, which are the most grown in greenhouse and open air in our country, were used.

2.2. Collecting flower buds and transferring them to the regeneration medium

The anthers of *Solanum melongena* (bitter yellow to yellow, that is, the period when the petal appears 2 mm) were taken and sterilized at the last stage of the single-nucleated microspore (Figure 1.). The collected buds were kept in 70% ethyl alcohol for 5 minutes in a sterile cabinet, then in 1% active sodium hypochlorite for 15 minutes and rinsed 3 times with sterile pure water. Sterilized anthers (3 different 2,4 D Kinetin and sucrose doses of the C medium reported in Dumas de Vaulx et al. (1982) were tested) were placed in the C medium whose contents are given in Table 1-2 and kept in the incubator in the dark at 35 ^{0}C for 8 days. Then, the petri dishes were placed under 16-hour light/8-hour dark conditions at 25 ^{0}C. Anthers were transferred from C medium to R medium after the 13th day. When embryos or plants were formed in R medium, they were transferred to V3 medium. Observations and counts of callus and plant development were made from anthers. (Figure 2.). The resulting plants were transferred to glass jars containing V3 medium before acclimating them to the external environment. Then, the developing plants were planted in closed plastic boxes of 40x60x30 cm (width, height, width) by spraying them with a perlite-peat mixture (1/1 v/v) fungicide (Figure 3.). The plastic boxes were kept in the growth room for 1 week and gradually acclimated to external conditions in greenhouses with a misting system.

Solanum melongena cv Aydın Siyahı *Solanum melongena* cv Kemer

Figure 1. Selection of suitable buds for anther culture in *Solanum melongena*

Table 1. Contents of C, R, V3 nutrient media (mg/L)

	C	R	V3		C	R	V3
Macro nutrients				**Vitamin and amino acids**			
KNO_3	2150	2150	1900	Myo-inositol	100.00	100.00	100.00
NH_4NO_3	1238	1238	1650	Pyrodoxin HCl	5.500	5.500	5.500
$MgSO_4$-$7H_2O$	412	412	370	Nicotinic acid	0.700	0.700	0.700
$CaCl_2$-$2H_2O$	313	313	440	Thyamine HCl	0.600	0.600	0.600
KH_2PO_4	142	142	170	Calcium panthotenate	0.500	0.500	0.500
$Ca(NO_3)_2$-$4H_2O$	50	50	-	Vitamin B_{12}	0.030	-	-
NaH_2PO_4-H_2O	38	38	-	Biotin	0.005	0.005	0.005
$(NH_4)_2SO_4$	34	34	-	Glycin	0.100	0.100	0.200
KCl	7	7	-				
Micro nutrients				**Chelated Irons**			
$MnSO_4$-H_2O	22.130	20.130	0.076	Na_2-EDTA	18.65	18.65	37.30
$ZnSO_4$-$7H_2O$	3.625	3.225	1.000	$FeSO_4$-$7H_2O$	13.90	13.90	27.80
H_3BO_3	3.150	1.550	1.000				
KI	0.695	0.330	0.010				
Na_2MoO_4-$2H_2O$	0.188	0.138	-				
$CuSO_4$-$5H_2O$	0.016	0.011	0.030				
$CoCl_2$-$6H_2O$	0.016	0.011	-				
$AlCl_3$-$6H_2O$	-	-	0.050				
$NiCl_2$-$6H_2O$	-	-	0.050				

Table 2. Plant growth regulators and their concentration

Growing medium	Plant growth regulators		Sucrose	Agar
	2.4-D	Kinetin		
	5*	5*	120**	7**
C	1*	1*	120**	7**
	0.01*	0.01*	30**	7**
R	-	0.01*	30**	7**
V3	-	-	30**	7**

* mg/L, ** g/L

2.3. Ploidy level analysis

Leaf samples from plants obtained from anther culture studies (80 plants, including the control plant) were ranked as ploidy with the flow cytometry device at Alata Horticultural Research Institute, according to Tuna (2014). Flow cytometry analyzes were performed in accordance with the kit procedure of the relevant device. Approximately 15 µg of tissue taken from young leaves of plants consisting of anthers was used. Plant tissues were chopped thoroughly with a razor blade after adding 0.5 ml of chopping buffer (Partec HR-A) using one-sided scalpels to remove cell nuclei. Chopped samples were filtered with a 30 µm permeability filter, then

Partec HR-B solution containing DAPI (4',6-diamidino-2-phenylindole) was added and incubated at room temperature for 5 minutes. As a result of incubation, the nuclear DNA content of the DAPI-stained nuclei of the leaves of the Kemer eggplant variety belonging to the control application was measured by UV LED-induced nuclear fluorescence at a wavelength of 365 nm in the Partec CyFlow Space flow cytometer. For each leaf sample, 1000-3000 nuclei were analyzed. The ploidy levels of the samples were determined by comparing them with the average radiation intensity value given by the reference nuclear DNA amount. (Figure 4.).

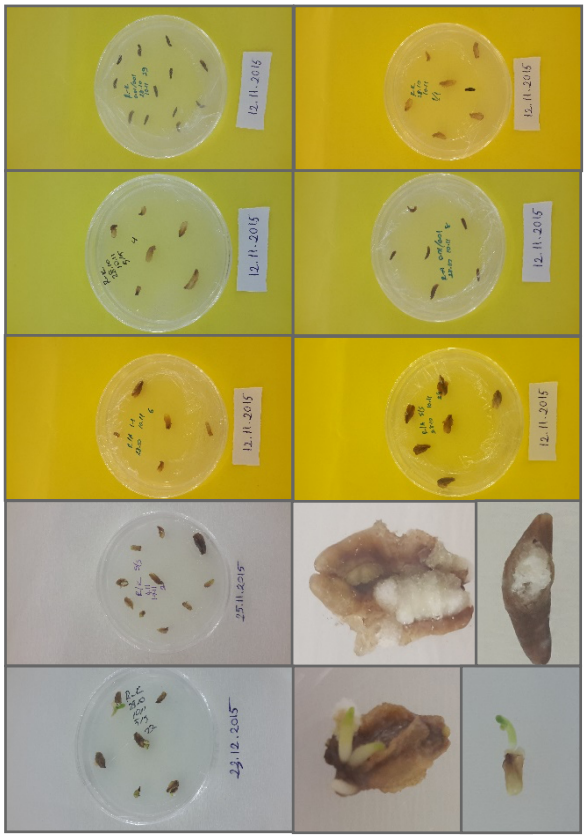

Figure 2. Plant development from anthers

Figure 3. Transferring plants consisting of anthers to jars, outdoors and land

Figure 4. Flow cytometry analysis

3. Results

In the anther culture studies in the fall of 2015, the anther development rate of the Aydın Siyahı variety was 75.2% and that of the Kemer variety was 72.6% in C (callus) medium to which 0.01 mg/l 2.4-D + 0.01 mg/l

Kinetin + 30 g/l sucrose was added. The highest callus development among the cultivated antes was 3.3% in the Aydın Siyahı variety, while it was 2.1% in the Kemer variety. Similarly, the highest embryo formation (4.23%) and plant yield (3.78%) were obtained from the Aydın Siyahı variety. (Table 3.).

In C (callus) medium with 1 mg/l 2.4-D + 1 mg/l Kinetin + 120 g/l sucrose, the anther development rate of the Aydın Siyahı variety was 82.9% and that of the Kemer variety was 77.9%. Callus development of the Aydın Siyahı variety was 34.7%, and that of the Kemer variety was 21.6%. The highest embryo formation (54.2%) and plant yield (36.4%) were obtained from the Aydın Siyahı variety. The embryo formation rate of the Kemer variety in this medium was found to be 21.5% and the plant formation rate was 14.1%. (Table 4.).

It was determined that the anther development rate of Aydın Siyahı variety was 85.5% and Kemer variety was 78.6% in C (callus) medium supplemented with 5 mg/l 2.4-D + 5 mg/l Kinetin + 120 g/l sucrose. Callus development rate was 53.1% for Aydın Siyahı variety and 34% callus rate was obtained for Kemer variety. The highest embryo formation (35.7%) and plant yield (38.8%) were obtained from the anthers of the Kemer variety. It was determined that the embryo formation rate of the Aydın Siyahı variety in 5/5 medium was 29.1% and the plant formation rate was 28.1%. (Table 5.).

High success was achieved in both direct plant formation (Figure 5.) and indirect plant formation (Figure 6.) from anthers taken from eggplants grown in the greenhouse during October in the 2015 fall period. The number of plants obtained from anther culture transferred to vials was 501, and the number of plants transferred to the field was 90 (Table 6.). While no plants were obtained from the Kemer variety in the 0.01/0.01 application, 19 plants from the Aydın Siyahı variety were transferred to *in vitro* medium acclimatization vials and 6 of them were transferred to the greenhouse. The highest number of plants transplanted into both the viols (171 plants) and the field (27 plants) was obtained from the 1/1 application of the Aydın Siyahı variety. This was followed by 5/5 application of the Kemer variety (124 plants to the viols and 22 plants to the field, respectively).

The ploidy level obtained from anther culture was determined as 60 of the 79 plants examined, 60 as haploid, 13 as diploid, 4 as triploid, 1 as tetraploid and 1 as mixoploid. Of the plants whose ploidy level was determined, 26 of them were obtained from Aydın Siyahı 1/1, 20 from Aydın Black 5/5, 23 from Kemer 5-5, 8 from Kemer 1/1 and 2 from Aydın Siyahı 0.01/0.01 application.

Table 3. Results obtained from anther culture on 0.01/0.01 medium

Varieties	Number of cultured anthers	Anther development		Frequency of calli formation		Frequency of embryo formation		Plant formation	
		Number	%	Number	%	Number	%	Number	%
Aydın Siyahı	899	676	75.2	22	3.3	38	4.23	34	3.78
Kemer	1338	971	72.6	28	2.1	17	1.27	1	0.07

Table 4. Results obtained from anther culture on 1/1 medium

Varieties	Number of cultured anthers	Anther development		Frequency of calli formation		Frequency of embryo formation		Plant formation	
		Number	%	Number	%	Number	%	Number	%
Aydın Siyahı	936	776	82.9	325	34.7	507	54.2	341	36.4
Kemer	1290	1005	77.9	278	21.6	277	21.5	182	14.1

Table 5. Results obtained from anther culture on 5/5 medium

Varieties	Number of cultured anthers	Anther development		Frequency of calli formation		Frequency of embryo formation		Plant formation	
		Number	%	Number	%	Number	%	Number	%
Aydın Siyahı	922	788	85.5	490	53.1	268	29.1	171	28.1
Kemer	1323	1040	78.6	450	34.0	472	35.7	394	33.8

Table 6. Transferring plants obtained from anther culture to the external environment

Varieties	C medium	Number of plants transferred to vials	Number of plants transferred to the greenhouse
Aydın Siyahı	**0.01/0.01**	19	6
	1/1	171	27
	5/5	115	22
Kemer	**0.01/0.01**	-	-
	1/1	72	13
	5/5	124	22
Total		**501**	**90**

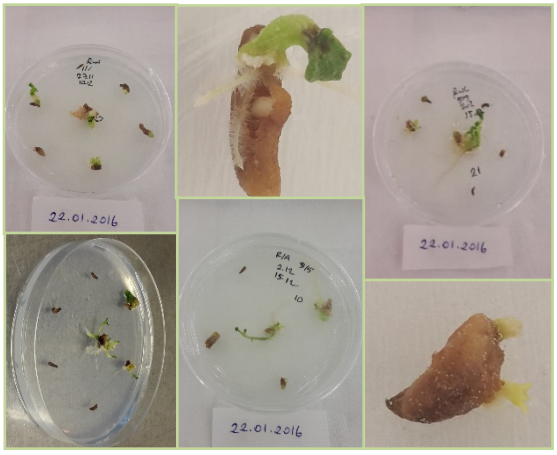

Figure 5. Direkt androgenesis

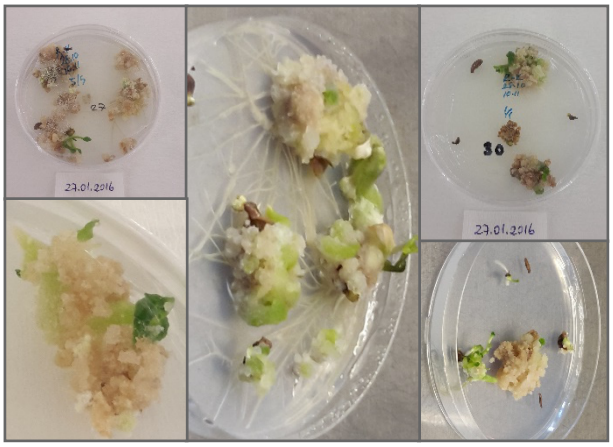

Figure 6. Indirect androgenesis

4. Discussion

Başay et al. (2010) determined that anther culture resulted in 14.29% embryo formation in the Bonica F1 variety, while anther culture was not successful in *S. torvum* and *S. sodomeum* species. They also determined that, in addition to the genotype effect in anther culture, the growing conditions of the donor plant are an important factor affecting the result obtained from anther culture. Alpsoy and Şeniz (2007) obtained 3.67% of plants from the Urfa Yerlisi variety by anther culture. Başay et al (2011) claimed that embryo formation was higher in anther culture established with buds obtained from eggplants grown in summer.

In previous studies carried out to establish an anther culture protocol in eggplant, although callus was obtained, transformation into plants and haploid plant production could not reach the desired level. It should be said that the factors affecting the success of anther culture in eggplant are the variety, the growing season of the donor plant and, perhaps most importantly, the choice of growth regulator and sucrose dose in the regeneration medium. Because the anthers placed in the same growing environment under the same conditions could not turn into plants only because the amount of hormones and sucrose were lower. While 394 plants of Kemer variety were obtained in the medium containing 5 mg/L 2.4-D + 5 mg/L Kinetin + 120 g/L

sucrose, 0.01 mg/L 2.4-D + 0.01 mg/L Kinetin + 30 g/L The fact that only one plant from the Kemer variety was obtained in the medium containing sucrose proves this.

Embrogenic calli formed in anther culture are valuable as a source of somatic hybridization between species. Kashyap et al. (2003) reported that protoplast fusion used in somatic hybridization was used to transfer beneficial traits in interspecific hybridization in case of sexual incompatibility in eggplant. They emphasized that one of the most important advantages of the somatic hybrid is that, in addition to nuclear properties, cytoplasmic properties, which are of economic importance, can be transferred to the hybrid. In this context, embrogenic calli obtained from anther culture will allow somatic hybridization in interspecies hybridizations.

Funding: This research was funded by Cukurova University BAP, grant number FBA-2015-3552.

Conflicts of Interest: The author declare no conflict of interest.

References

Alpsoy HC, Şeniz V (2007). Researches on the *in vitro* androgenesis and obtaining haploid plants in some eggplant genotypes. *Acta Horticulturae* 729: 137–141.

Başay S, Şeniz V, Ellialtıoğlu ŞŞ (2011). Obtaining dihaploid lines by using anther culture in the different eggplant cultivars. *Journal of Food, Agriculture & Environment* 9: 188-190.

Başay S, Ellialtıoğlu ŞŞ, Şeniz V (2010). Haploid embryo formation via anther culture in local and foreign eggplant varieties. VIII. *Vegetable Agriculture Symposium*, 23-26 June 2010, Van, Turkey. pp.:588-590.

Calabuig-Serna A, Porcel R, Corral-Martínez P, SeguíSimarro JM (2020). Anther culture in eggplant (*Solanum melongena* L.). In: Plant Embryogenesis. *Methods in Molecular Biology*, Volume 2122, Humana Press, New York, 283293.

Dumas De Vaulx R, and Chambonnet D (1982). Stimulation of plant production in eggplant (*Solanum melongena* L.) anther culture by treatment at +35°C and low growth substance concentrations. *Agronomie* 2 (10): 983-988.

Ellialtıoğlu ŞŞ, Başay S, Kuşvuran Ş (2012). Investigation of the relationship between pollen dimorphism and anther culture in eggplant. *Journal of Agricultural Sciences Research* 5(1): 149-152.

FAO (2024). Production and trade statistics. https://www.fao.org/faostat/en/#data/QCL (access date: 11.11.2024).

Gebeloğlu N, Ellialtıoğlu ŞŞ (2022). Eggplant breeding. *Vegetable breeding, vol. III. Solanaceae,* Gece publishing, Türkiye, pp. 319-412.

Kashyap V, Vinod Kumar S, Collonnier C, Fusari F, Haicour R, Rotino GL, Sihachakr D, Rajam MV (2003). Biotechnology of Eggplant. *Scientia Horticulturae* 97(1): 1-25.

Khatun F, Meah MB, Nasiruddin KM (2006). Regeneration of eggplant through anther culture. *Pakistan Journal of Biological Sciences* 9: 48–53.

Kumar S, Singh M, Prabhavathi K, Mathews A, Kumar S, Singh M, Mathews A (2003). *In vitro* induction of haploid in eggplant (*Solanum melongena* L.). *Capsicum* and *Eggplant Newsletter* 22: 147–150.

Mir R, Calabuig-Serna A, Seguí-Simarro JM (2021). Doubled haploids in eggplant. *Biology* 10(7): 685.

Salas P, Prohens J, Seguí-Simarro JM (2011). Evaluation of androgenic competence through anther culture in common eggplant and related species. *Euphytica* 182: 261–274.

Salas P, Rivas-Sendra A, Prohens J, Seguí-Simarro JM (2012). Influence of the stage for anther excision and heterostyly in embryogenesis induction from eggplant anther cultures. *Euphytica* 184(2): 235–250.

Seguí-Simarro JM (2016). Androgenesis in *Solanaceae* (M. Germana, and M. Lambardi editors). *In Vitro* embryogenesis in higher plants. *Methods in Molecular Biology*, vol 1359. Humana Press, New York, NY, 206–244.

Tuna M (2014). 2nd workshop on flow cytometry and its use in agricultural research. *Namık Kemal University Faculty of Agriculture, Department of Field Crops*,16-17 January 2014, Tekirdağ.

Vural GE, Ari E, Zengin S, Ellialtioglu SS (2019). Development of androgenesis studies on eggplant (*Solanum melongena* L.) in Turkey from past to present. *In: Sustainable Crop Production, IntechOpen*, London, 67–92.