Gaziosmanpaşa Üniversitesi Ziraat Fakültesi Dergisi
Journal of Agricultural Faculty of Gaziosmanpasa University
http://ziraatdergi.gop.edu.tr/

JAFAG
ISSN: 1300-2910
E-ISSN: 2147-8848
(2020) 37 (1), 23-29
doi:10.13002/jafag4627

Araştırma Makalesi/*Research Article*

Effect of Media, Stress Conditions and Genotype on Androgenetic Performance of Pepper

Sevtap Doksöz Boncukçu [1*], Murat Emre Çelik[1], Naif Geboloğlu[1]
Tokat Gaziosmanpaşa University, Agricultural Faculty, Horticulture Department- Tokat
(orcid.org/0000-0001-9938-3790); (orcid.org/0000-0002-7003-878X); (orcid.org/0000-0003-2495-7088)
* e-mail: sevtap.doksoz@gop.edu.tr

Alındığı tarih (Received): 26.06.2019 Kabul tarihi (Accepted): 30.03.2020
Online Baskı tarihi (Printed Online): 15.04.2020 Yazılı baskı tarihi (Printed): 30.04.2020

Abstract: This study was conducted with the purpose of investigation of the effects of genotype, nutrient media, pre-treatments and incubation conditions on haploid plant development on some three-lobed pepper genotypes. İstek F_1, Köylüm F_1, Üçburun F_2 and Tokat local genotype were used as donor plants. DDV (Dumas de Vaulx et al., 1981) and MS (Murashige and Skoog, 1962) were used as nutrient media. Furthermore, 0.01 mg L^{-1} Kinetin + 0.01 mg L^{-1} 2,4-D + 0.03 mg L^{-1} Vitamin B12 were added to the media. Flower buds were held at 4 ºC for 24 hours. Low-temperature treatment was not applied on flower buds of control plants. After anthers were placed in the nutrient media, they were held at 9 ºC and 35 ºC for 8 hours in the darkness. Afterwards, anthers were held at 25±2 ºC for 16 h day / 8 h night for 4 days and transferred to regeneration media. All genotypes that were used in the experiment gave a response to the anther culture more or less. The highest embryo development was obtained from Tokat genotype. Embryo development rates varied between 0.25% and 31.00% by treatments. While embryo development rate was 3.9% in MS medium, it was 9.33% in DDV medium. Moreover, while embryo development rate was 6.7% on anthers that were exposed to cold pre-treatment, it was 6.4% in the control treatment. Embryo development rate in 9 ºC and 35ºC incubation treatments were 7.60% and 5.50% respectively.

Keywords: Anther culture, DDV, genotype, incubation, MS, pre-treatment

Besin Ortamı, Stres Koşulları ve Genotipin Biberde Androgenik Başarı Üzerine Etkisi

Öz: Çalışmada üç burun meyve yapısına sahip biber genotiplerinde genotip, besi ortamı, stres ve inkübasyon uygulamalarının anter kültürü yöntemiyle haploid bitki oluşumuna etkileri araştırılmıştır. Denemede donör bitki olarak İstek F_1, Köylüm F_1, Üçburun F_2 ve yerel genotip olan Tokat biberi kullanılmıştır. DDV (Dumas de Vaulx ve ark., 1981) ve MS (Murashige ve Skoog, 1962) olarak bilinen ortamlar besi ortamı olarak kullanılmıştır. Ortamlara 0.01 mg/L Kinetin + 0.01 mg/L 2.4D + 0.03 mg/L Vitamin B12 ilave edilmiştir. Çiçek tomurcukları 4 ºC'de 24 saat bekletilmiştir. Kontrol bitkilerinin tomurcuklarına düşük sıcaklık uygulaması yapılmamıştır. Anterler, besi ortamına ekildikten sonra 9 ºC ve 35 ºC'de 8 gün süreyle karanlık ortamda bekletilmiş ardından 16 saat gündüz/8 saat gece ve 25±2 ºC sıcaklığa sahip ortama alınmıştır. Bu ortamda 4 gün bekleyen anterler daha sonra rejenerasyon ortamına aktarılmıştır. Denemede kullanılan genotiplerin hepsi anter kültürüne az ya da çok cevap vermişlerdir. En yüksek embriyo oluşumu yerel Tokat genotipinden elde edilmiştir. Embriyo oluşum oranları uygulamalara göre % 0.25 ile % 31.00 arasında değişmiştir. MS besi ortamına ekilen anterlerden embriyo oluşum oranı %3.9 olurken, DDV ortamına ekilen anterlerden embriyo elde etme oranı % 9.33 olmuştur. Ön uygulamaya tabi tutulan anterlerde embriyo oluşum oranı %6.7 olurken, kontrol uygulamasında % 6.4 olmuştur. Çalışmada 9 ºC inkübasyon uygulamasında embriyo oluşum oranı % 7.60 olurken, 35 ºC'de inkübasyon uygulamasında embriyo oluşum oranı % 5.50 olmuştur.

Anahtar Kelimeler: Anter kültürü, DDV, genotip, inkübasyon, MS, ön uygulama

1. Introductıon

Capsicum annuum is the most produced pepper among all species and has the biggest share of pepper production in Turkey and in the world. Pepper production increases continuously between 4-10% every year in Turkey. Especially since the green revolution, there have been important developments in pepper like many species, and breeding studies increased. Pepper breeding has been developed in all-purpose, and many superior biotechnological methods have been started to use. Anther culture is significant among these methods. The first haploid plant developed from male gamete was found in a

cotton plant in 1920 (Dunwell, 2010). In addition, first studies about haploid plant development in *in vitro* conditions with anther culture method were started by Wang et al. (1973). The first pollen embryogenesis studies were started by George and Narayanaswamy (1973). In Turkey, Abak (1983) has led first studies about anther culture. 10.38% success rate was obtained with proper nutrient media in pepper originated from Turkey (Abak, 1983). Taşkın (2005) indicated that genotype had a considerable effect on anther development in 5 different pepper genotypes with 4 different nutrient media. Çiner and Tıpırdamaz (2002) stated that the most proper stage for anther culture is calyx and corolla levels of flower buds are in same length or corolla is a slightly longer than calyx. Mityko et al. (1995) also indicated that the most proper stage for anther culture is corolla and calyx are in the same length or corolla is a bit longer. Sayılır and Özzambak (2000) determined optimum microspore stage when buds reached to 4-6 mm length. Çömlekçioğlu et al. (2001) reported that optimum stage for anther culture is when anthocyanin developed in half of the anthers. Moreover, the optimum stage for anther culture was determined by Chambonnet (1988) that when microspores were in mitosis-I phase. In addition, it was emphasized that temperature degree and duration in cold pretreatment is important, pretreatments at temperate temperatures like between 7-15 °C for 7-14 days were found more effective than pretreatments at low temperatures for short periods (Sunderland and Roberts, 1979). Sayılır and Özzambak (2002) reported that holding buds at 4 ºC for 25 hours before culturing of anthers gave effective results. Sayılır and Özzambak (2002) researched effects of nutrient media that were Murashige and Skoog, and Nitsch modified with NAA, BA, activated charcoal and carrot extract. Activated charcoal and carrot extract did not have a significant effect, moreover, the best result was obtained from MS with NAA and BA. Abak (1984) got most successful results in Turkey originated pepper from nutrient media with 5 mg/l kinetin, 5 mg L^{-1} 2.4-D, 120 g L^{-1} saccharose, 37.3 g L^{-1} Na_2EDTA+27.8 mg L^{-1} $FeSO_4.7$ H_2O. Ellialtıoğlu et al. (2001) got the highest embryo development rate in pepper with 1% from anthers cultured in MS medium with activated charcoal and carrot extract. Ercan et al. (2001) studied with 5 different pepper varieties and 11 different nutrient media (with different amount of kinetin, BA, NAA, 2,4-D, activated charcoal), and the best results were obtained from MS medium with 1% activated charcoal, 5 mg L^{-1} 2.4-D, 5 mg L^{-1} kinetin, and MS medium with 1% activated charcoal, 4 mg L-1 NAA + 0.1 mg L^{-1} BA. Furthermore, Alremi et al. (2013) reported that MS medium with 4 mg L^{-1} mg L^{-1} NAA + 0.1 mg L^{-1} BAP had significant effects on androgenic embryo development in pepper (*Capsicum annuum* L.).

In the present study, effects of genotype, nutrient media, stress and incubation treatments on haploid plant development with anther culture method in some pepper varieties were investigated.

2. Materıal and Methods

The study was conducted in the laboratory and research field belongs to Gaziosmanpaşa University, Faculty of Agriculture, Department of Horticulture. Screen house that is suitable for soilless culture was used with the purpose of growing donor plants. 2 hybrid (İstek F1, Köylüm F1), 1 F_2 (Üçburun F_2) and 1 local (Tokat pepper) three-lobed pepper genotypes were used as plant material. The seeds belong to pepper genotypes were sowed in the peat in multiple pods. Seedlings were planted in cocopeat blocks under the screen house. Flower buds were harvested when corolla was slightly higher than calyx. In this period, anthocyanin development occurred in 25% of anthers. This stage involves last phases of mononuclear microspore development and primary phase of pollen mitosis-I. Flower buds were held at 4 °C for 24 hours before disinfection. Low-temperature treatment was not applied in control plants. Flower buds with anthers that have the most suitable microspore development stage for pollen embryogenesis, were disinfected for 15 minutes with 20% commercial sodium hypochlorite added with few a drops of Tween-20. Afterwards, they were rinsed three times with distilled water. Anthers were cultured in DDV

(Dumas de Vaulx et al., 1981) and MS (Murashige and Skoog, 1962*)* media with 0.01 mg L^{-1} Kinetin + 0.01 mg L^{-1} 2,4D + 0.03 mg L^{-1} Vitamin B12. After anthers were placed in nutrient media, they were held at 9 °C and 35 °C for 8 days in the darkness. Afterwards, anthers were held at 25±2 ºC for 16 h day / 8 h night for 4 days and transferred to regeneration media. Embryoids that were developed from anthers, were transferred to the glass tubes in sterile conditions. When plantlets that were developed from embryoids in the glass tubes were at 3-4 leafed stage, they were transferred in ½ peat + ½ pearlite media with the purpose of acclimatisation to outside conditions.

3. Results and Dıscussıon

4800 anthers were planted in total from 4 genotypes. There were development and growth on anthers of all genotypes. The number of anthers that were cultured, number of the developed embryo, and number of developed plant from embryo were recorded by continuous observations. The highest embryo development rate was obtained with 34% from Tokat pepper that was incubated at 9 °C without cold pretreatment in MS medium (Figure 1). In İstek F_1 genotype, the highest embryo development was obtained with 28% with 4 ºC pretreatment for 24 hours and 9 ºC incubation treatment in DDV medium. It was followed with 26% by Tokat pepper that was exposed to 4 ºC pretreatment for 24 hours and 35 ºC incubation treatment in DDV medium (Table 1). Moreover, there was no androgenic response on anthers belong to Köylüm F_1 and Üçburun F_2 that were cultured in MS medium. Similarly, there was no development in Üçburun F_2 that was incubated at 9 ºC in DDV medium, in İstek F_1 that was not exposed to cold pretreatment and incubated at 9 ºC in MS medium, and in İstek F_1 that was exposed to cold pretreatment and incubated at 35 ºC in MS medium. Furthermore, the highest rate of plant development from anthers belongs to İstek F_1 variety with 5.33% that was treated with 4 ºC/24 h cold pretreatment and 9 ºC incubation treatment in DDV medium.

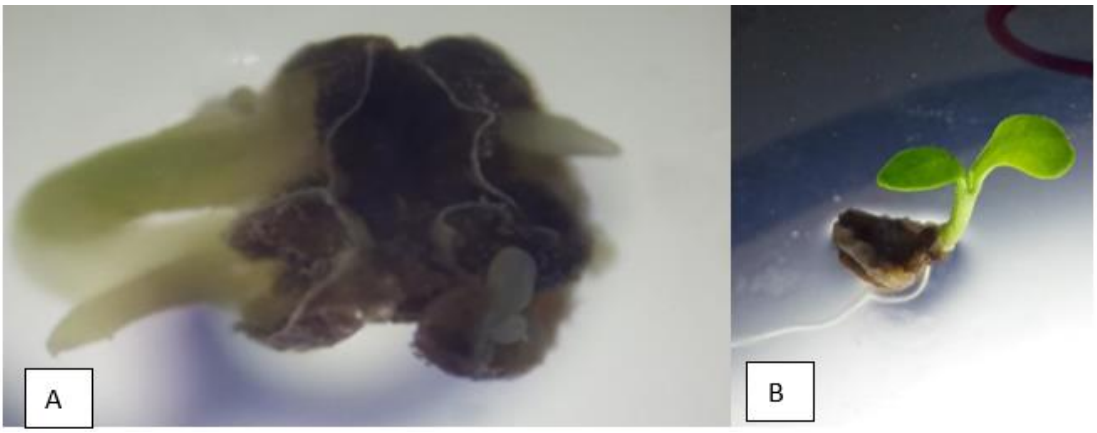

Figure 1. A Cultured anther, open along the dehiscence line, through which microspore-derived embryo is emerging. **B** Young seedling directly emerged from the anther locule.

Şekil 1. A *Kültüre alınan anterlerde mikrospolardan embriyo gelişimi* ***B*** *Anterlerden direk bitkicik oluşumu*

Table 1. Embryoid and plant development rates according to genotype, nutrient media, stress and incubation conditions

Tablo 1. *Genotip,besin ortamı,stres ve inkübasyon koşullarına göre embriyo ve bitki gelişim oranları*

Media	Genotype	Pretreatment (holding at 4°C)	Incubation treatments(°C)	Anther number	Embryo number	The rate of embryo development (%)	Plant number	The rate of plant development from embryo (%)
DDV	İstek F_1	Control	9	150	6	4,00	5	83,33
	İstek F_1	Control	35	150	12	8,00	3	25,00
	İstek F_1	24 h	9	150	42	28,00	8	19,04
	İstek F_1	24 h	35	150	12	8,00	7	58,33
	Tokat Pepper	Control	9	150	39	26,00	4	10,25
	Tokat Pepper	Control	35	150	10	6,66	1	10,00
	Tokat Pepper	24 h	9	150	18	12,00	7	70,00
	Tokat Pepper	24 h	35	150	39	26,00	3	7,70
	Köylüm F_1	Control	9	150	3	2,00	0	0,00
	Köylüm F_1	Control	35	150	3	2,00	0	0,00
	Köylüm F_1	24 h	9	150	3	2,00	1	33,33
	Köylüm F_1	24 h	35	150	6	4,00	4	66,6
	Üçburun F_2	Control	9	150	0	0,00	0	0,00
	Üçburun F_2	Control	35	150	10	6,66	3	30,00
	Üçburun F_2	24 h	9	150	0	0,00	0	0,00
	Üçburun F_2	24 h	35	150	7	4,66	1	14,28
MS	İstek F_1	Control	9	150	0	0,00	0	0,00
	İstek F_1	Control	35	150	10	6,66	2	20,00
	İstek F_1	24 h	9	150	4	2,66	1	25,00
	İstek F_1	24 h	35	150	0	0,00	0	0,00
	Tokat Pepper	Control	9	150	51	34,00	4	7,84
	Tokat Pepper	Control	35	150	10	6,66	0	0,00
	Tokat Pepper	24 h	9	150	6	4,00	2	33,30
	Tokat Pepper	24 h	35	150	14	9,30	1	7,14
	Köylüm F_1	Control	9	150	0	0,00	0	0,00
	Köylüm F_1	Control	35	150	0	0,00	0	0,00
	Köylüm F_1	24 h	9	150	0	0,00	0	0,00
	Köylüm F_1	24 h	35	150	0	0,00	0	0,00
	Üçburun F_2	Control	9	150	0	0,00	0	0,00
	Üçburun F_2	Control	35	150	0	0,00	0	0,00
	Üçburun F_2	24 h	9	150	0	0,00	0	0,00
	Üçburun F_2	24 h	35	150	0	0,00	0	0,00

In Tokat pepper, the success rate of plant development from anthers reached 4.66% with 4 ºC/24 h cold pretreatment and 9 ºC incubation treatment in DDV medium. Moreover, the highest rate of plant development from embryos was obtained from İstek F_1 variety with 83.33% that was incubated at 9 ºC without cold pretreatment, and it was followed by Köylüm F_1 genotype with 66.60% that was treated with cold pretreatment (4 ºC/24 h) and incubated at 35 ºC. There were significant differences between genotypes according to androgenesis success. When general performances of genotypes are observed, Tokat pepper and İstek F_1 gave the best results.

The success rate of DDV medium was found higher. Performances of nutrient media vary by the other factors also. Matsubara et al. (1992) used different combinations of MS medium and obtained 8% embryo development. Ellialtıoğlu et al. (2001) studied with Kahramanmaras pepper in MS medium with 4 mg L^{-1} NAA and 0.1 mg L^{-1} BAP, they determined 2.28% embryo development rate as a result. Çömlekçioğlu et al. (2001) used the same method, however, there was no embryo development. Koleva-Gudeva (2007) used MS (Murashige and Skoog, 1962), N (Nitsch, 1974), LS (Linsmaer and Skoog, 1965), NN (Nitsch and Nitsch, 1969) and CP (Dumas de Valux et al., 1981) media in the study about the effects of incubation treatments and different nutrient media on haploid plant development on pepper with anther culture. As a result, higher success was obtained from MS and CP media. While embryo development was 7.60% at 9 ºC incubation treatment, it was 5.50% at 35 ºC incubation treatment. Researchers generally prefer incubation conditions in anther culture studies as 9 ºC or 35 ºC for 7 or 8 days in the darkness (Ellialtıoğlu et al., 2001). Terzioğlu et al. (2000) stated that incubating anthers at high-temperature is effective on haploid plant development. Ellialtıoğlu et al. (2000) stated that in the first days after cultivation of anthers, high-temperature treatments on pepper and eggplant had positive effects. Koleva-Gudeva (2007) experienced incubation treatments at 7º C, 25º C, and 35º C on anthers of nine different pepper genotypes. As a result, while 30.6% of success was obtained at 25ºC, there was no development on four in nine genotypes at 35º C. Most of the researchers suggest high-temperature in incubation treatment studies. However, few researchers stated that there has been no development on high-temperature incubation treatments. In the present study, even there was a higher success rate at 9 ºC incubation conditions, high results were obtained from İstek F_1 genotype that was incubated at 35 ºC without cold pretreatment, and Tokat pepper that was incubated at 35 ºC with cold pretreatment. Hence, high success rates that have been indicated in previous studies, were obtained in the present study as well.

4. Conclusıon

The present study investigated the effects of genotype, nutrient media, stress and incubation treatments on haploid plant development on four different genotypes. Embryoid development rates varied between 0.66-34.00% when unsuccessful treatments are not considered. The rate of plant development from anthers was between 0.66-5.33%, while the rate of plant development from embryos was between 7.70-83.33%. Therefore, the effects of treatments can be observed clearly with these results. Besides unsuccessful treatments, positive results were obtained according to genotype and other treatments. As a conclusion, Tokat pepper that is a local genotype showed the best androgenic performance followed by İstek F_1 among all genotypes. While holding flower buds at 4 ºC for 24 h was found more effective among pretreatments, effect of incubation treatments showed differences according to treatment. Furthermore, higher results were obtained from DDV medium than MS medium.

References

Abak K (1983). Study on the anther culture in vitro of pepper (*Capsicum annuum*). Capsicum Newslett, *2*, 72-73.

Abak K (1984). Biberde (*Capsicum annuum* L.) anter kültürü yolu ile haploid bitki elde etme üzerinde

araştırmalar. Ankara Üniversitesi. Ziraat Fak Yıllığı. 1–4, 155–163 (in Turkish).

Achar PN (2002). A study of factors affecting embriyo yields from anther culture of cabbage. Plant Cell Tissue Organ Cult. *69*(2), 183-188.

Alremi F, Taskın H, Sonmez K, Buyukalaca S, Ellialtıoglu S (2013). Biber (*Capsicum annuum* L.)'de Genotip ve Besin Ortamının Anter Kültürüne Etkileri DOI: 10.30910/turkjans.160702.

Cardoza V, Stewart JCN (2004). Brassica biotechnology: progress in cellular and molecular biology. In Vitro Cel Dev Biol Plant. *40*(6), 542-551.

Chambonnet D (1988). Production of haploid pepper plants. Bulletin interne de la station d'Amelioration des Plantes Maraicheres d'Avignon-Montfavet, France. 1-10.

Chlyah A, Taarji H (1984). Proc. Int. Symp. Plant tissue and cell culture application to crop improvement. 24–29 Sept.

Ciner D, Tıpırdamaz R (2002). The effects of cold treatment and charcoal on the *in vitro* androgenesis of pepper (*Capsicum annuum* L.). Turkish Journal of Botany. *26*,131-139.

Comlekcioglu N, Büyükalaca S, Abak K (2001). Effect of silver nitrate on haploid embryo induction by anther culture in pepper (*Capsicum annuum*). XIth EUCARPIA Meeeting on Genetics and Breeding of Capsicum & Eggplant, 2001, Antalya-Turkey

Dumas De Vaulx R, Chambonnet D, Sibi M (1981). Culture *in vitro* d'antheres de piment (*Capsicum annuum* L.): amelioration des taux d'obtention de plantes chez differents genotypes par de traitments A+35°C. Agronomie. 11, 859.

Dunwell JM (2010). Haploids in flowering plants: origins and exploitation. Plant Biotechnology Journal. *8*, 377–424.

Ellialtıoglu S, Sari N, Abak K (2000). Haploid Bitki Üretimi. (Bitki Biyoteknolojisi Cilt:I, Ed: Babaoğlu, M., Özcan, S., Gürel, E.) 40 s.

Ellialtıoglu S, Kaplan F, Abak K (2001). The effect of carrot extract and activated charcoal on the androgenesis of pepper. In Proceedings of XIth EUCARPIA Meeting on Genetics and Breeding of Capsicum & Eggplant. pp. 9-13

Ellialtıoglu S, Tıpırdamaz R (1997). Soğuk uygulamaları ve aktif kömürün patlıcan ve biberde in vitro androgenesis üzerine etkileri, TÜBİTAK-TOGTAG 87 No'lu proje Sonuç Raporu, 70 s. Ankara.

Ercan N, Boyacı F, Ayar F (2001). Biberde (*Capsicum annuum* L.) anter kültürü yoluyla haploid bitki eldesi üzerine farklı besin ortamlarının etkisi. GAP II. Tarım Kongresi, 24-26 Ekim, Şanlıurfa, Cilt 1: 121-128.

Foroughi-Wehr B, Wenzel G (1993). Andro- and parthenogenesis. In: Plant Breeding. Principles and Prospects. Hayvard, M.D., Bosemark, N.O., Ramagosa, I. (eds.)., Chapman & Hall, London, pp. 261-277.

George L, Narayanaswamy S (1973). Haploid Capsicum through experimental androgenesis. Protoplasma *78*, 467-470.

Koleva-GudevaL R, Spasenoski M, Trajkova F (2007). Somatic embryogenesis in pepper anther culture: The effect of incubation treatments and different media. Scientia Horticulturae. *111*(2), 114-119.

Kothari SL, Joshi A, Kachhwaha S, Ochoa-Alejo N (2010). Chilli peppers—a review on tissue culture and transgenesis. Biotechnology advances. *28*(1), 35-48.

Kristiansen K, Andersen SB (1993). Effects of donor plant temperature, photoperiod, and age on anther culture response of *Capsicum annuum* L. Euphytica. 67,105– 109.

Linsmaier EM, Skoog F (1965). Organic growth factor requirements of tobacco tissue cultures. Physiol. Plant. *18*,100±127.

Malhotra K, Maheshwari SC (1977). Enhancement by Cold Treatment of Pollen Embryoid Development in *Petunia hybrida* Zeitschrift für Pflanzenphysiologie. *85*(2), 177-180 https://doi.org/10.1016/S0044-328X(77)80293-6.

Matsubara S, Hu K, Murakami K (1992). Embryoid and callus formation from polen grains of eggplant and pepper by anther culture. Journal of the Japanese Society for Horticultural Science *61*(1), 69–77 https://doi.org/10.2503/jjshs.61.69

Mityko J, Andrasfalvy A, Csıllery G, Farı M (1995). Anther culture

response in different genotypes and F1 hybrids of pepper (*Capsicum annuum* L.). Plant Breeding, *114*,78-80.

Mityko J, Fari M (1997). Problems and results of doubled haploid plant production in pepper (*Capsicum annuum* L.) via anther and microspore culture. Acta Hortic. *447*,281-287 DOI: 10.17660/ActaHortic.1997.447.57

Morrison RA, Koning, RE, Evans DA (1986). Anther culture of ınterspesific hybrid of Capsicum. J. Plant Physiol. *126*,1-9.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassy with tobacco tissue cultures. Physiol. Plant. *15*, 473-497 https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Nitsch JP, Nitsch C (1969). Haploids Plants from Pgrains. Science *163*, 85-87.

Nitsch C (1974). La culture de pollen isole sur milieu synthetique. C. R. Acad. Sci. Ser. D., *278*, 1031-1034.

Ochoa-Alejo N, Ramirez-Malagon R (2001). In vitro chili pepper biotechnology. In Vitro Cel Dev Biol Plant. *37*(6), 701-729.

Rodeva V, Grozeva S, Todorova V (2006). In vitro answer of Bulgarian pepper (*Capsicum annuum* L.) varieties. Genetica (Serbia) *38*(2),129–136.

Sayılır A, Ozzambak ME (2000). Brokkolide anter kültürü denemeleri. III. Sebze Tarımı Sempozyumu, Isparta, 218-222.

Sayılır A, Ozzambak ME (2002). Biber anter kültüründe uygun tomurcuk büyüklüğü tespiti ile besin ortamları karışımlarının ve soğuk uygulama sürelerinin embriyo verimine etkisi üzerine bir araştırma. IV. Sebze Tarımı Sempozyumu, Bursa.

Smith RH (2013). Plant tissue culture: techniques and experiments. Academic Press.

Sunderland N, Roberts M (1979). Cold-pretremant of excised flower buds in float culture of tobacco anther. Ann. Bot. *43* (4),405-414 https://doi.org/10.1093/oxfordjournals.aob.a085650.

Taşkın H (2005). Different applications to improve the embryo quality on stimulation of embryo by anther culture in some pepper genotypes. Ziraat Fakültesi Yüksek Lisans, Sayfa Sayısı: 79.

Terzioglu S, Ellialtıoglu S, Abak K (2000). İnkübasyon koşullarının biber anter kültüründe embriyo oluşumu üzerine etkisi. III. Sebze Tarımı Sempozyumu.11-13 Eylül, Isparta. s:233-238.

Touraev A, Heberle-Bors E (2003). Anther and microspore culture in tobacco. In Doubled Haploid Production in Crop Plants (pp. 223-228). Springer Netherlands.

Wang YY, Sun, CS, Wang CC, Chien NJ (1973). The induction of polen plantlets of Triticale and *Capsicum annuum* from anther culture. Sci. Sin, *16*, 147-151.

Zhang E, Ou C, Xu Z, Cheng Y (2005). Factors affecting embryoid induction and formation of cabbage anthers in culture. Acta Botanica Boreali-Occidentalia Sinica. *26*(11), 2372