OKU Fen Bilimleri Enstitüsü Dergisi
6(3): 2095-2105, 2023

OKU Journal of The Institute of Science and Technology, 6(3): 2095-2105, 2023

Osmaniye Korkut Ata Üniversitesi Fen Bilimleri Enstitüsü Dergisi

Osmaniye Korkut Ata University Journal of The Institute of Science and Technology

Anther Culture Method on Haploid Plant in Pepper (*Capsicum annuum* L.)

Mevlüde TATAR[1*]
[1]Alata Horticultural Research Institute, 33740, Mersin, Türkiye

[1]https://orcid.org/0000-0002-3707-1721
*Corresponding author e-mail: mtatar65@hotmail.com

Research Article

Article History:
Received: 18.06.2022
Accepted: 19.12.2022
Published online: 04.12.2023

Key words:
Pepper
Anther culture
Haploid
Homozygous

ABSTRACT

Pepper is from the *Capsicum* genus of the *Solanaceae* family and is one of the vegetable species that is loved and grown in the world and in our country. After China and Mexico, Turkey ranks third in pepper production in the world. It is a great advantage for us that the climate structure of our country is suitable for vegetable cultivation. The adaptation of pepper to every region has led to the formation of a significant variety of pepper in the country in this way, it has also ensured the enrichment of our gene resources. The richness of gene resources is an important factor in the success of breeding studies. One of the purposes of breeding studies is to develop varieties. It is used in breeding studies with anther culture method in pepper, and new varieties are developed in this way. Because of obtaining homozygous lines in plants, haploid plant production has an important place in plant breeding. In this study, the definition and application of anther culture and the factors affecting success, the researches made with the anther culture method in the world and in our country and the developments in recent years are discussed.

Biberde (*Capsicum annuum* L.) Haploid Bitki Eldesinde Anter Kültürü Yöntemi

Araştırma Makalesi

Makale Tarihçesi:
Geliş tarihi: 18.06.2022
Kabul tarihi:19.12.2022
Online Yayınlanma: 04.12.2023

Anahtar kelimeler:
Biber
Anter kültürü
Haploid
Homozigot

ÖZ

Biber *Solanaceae* familyasının *Capsicum* cinsinden olup dünyada ve ülkemizde sevilerek tüketilen ve buna bağlı olarak yetiştirilen sebze türlerinden biridir. Biber Çin ile Meksika'dan sonra Türkiye dünyada üretiminde üçüncü sırada gelmektedir. Ülkemizin iklim yapısının sebze yetiştiriciliğine uygun olması bizim için büyük bir avantajdır. Bundan dolayı biberin her bölgeye uyum sağlamasıyla ülkede önemli oranda biber çeşitliliğinin olmasına neden olmuştur. Bu durum gen kaynaklarımızın zenginleşmesini de sağlamıştır. Islah çalışmalarının başarısını sağlayan en önemli faktörlerden biride gen kaynaklarının zenginliğidir. Islah çalışmalarının amaçlarından biride çeşit geliştirmektir. Biberde anter kültürü yöntemiyle ıslah çalışmalarında kullanılmakta bu yolla yeni çeşitler geliştirilmektedir. Bitkilerde homozigot hatların elde edilmesinden dolayı haploid bitki üretimi bitki ıslahında önemli bir yere sahiptir. Bu çalışma anter kültürünün tanımı, uygulaması ve başarıyı etki eden faktörlerle ilgili bilgiler ile dünyada ve ülkemizde anter kültürü yöntemiyle yapılan araştırmalar ve son yıllardaki gelişmeler ele alınmıştır.

To Cite: Tatar M. Anther Culture Method on Haploid Plant in Pepper (*Capsicum annuum* L.). Osmaniye Korkut Ata Üniversitesi Fen Bilimleri Enstitüsü Dergisi 2023; 6(3): 2095-2105.

1. Introductıon

In recent years, increasing population, rapidly developing urbanization, and the decrease in agricultural lands, changing climatic conditions now require faster, more precise, and short-term studies in breeding studies, and pure line/lines are obtained by anther culture method. This method is

the most preferred biotechnological method by researchers (Khush and Virmani, 1996; Çömlekçioğlu et al., 2001; Ellialtıoğlu and Tıpırdamaz, 2002; Popova et al., 2016; Parra-Vega and Segui-Simarro, 2016; Ozsan and Onus, 2017). The use of tissue culture techniques together in plant breeding is a great advantage for breeding studies, increasing genetic richness, and achieving more successful results in a shorter time period, and this has provided a wide application area in vegetable breeding. Haploid plant production, which is one of the tissue culture techniques, is very important in plant breeding (Andrews, 1985).

Anther culture, also known as pollen culture, is the technique of growing new plants by taking anthers isolated from a plant into a suitable food nutrient medium. It produces haploid (n chromosome) plants and is especially important in plant breeding. It is possible to select the desired mutant types and develop new cultivars with anther culture, which will provide a large amount of haploid plant production. It starts with removing the anthers from the flower buds during pollen formation and taking them into the nutrient media under aseptic (germ-free) conditions. Anthers develop into this culture media by undergoing mitosis. When they complete their development process, haploid plantlets are obtained (Heiser, 1976; Andrews, 1985; Abak, 1986; Khush and Virmani, 1996; Ellialtıoğlu et al., 2006; Olszewska et al., 2014; Arı et al.,2016).

Haploid plants are important materials used in genetic analysis and similar studies in breeding studies. In order for haploid plants to be used in breeding studies, anther culture is preferred so that they can be obtained from the desired genetics when desired in sufficient quantity and easily. Chromosome doubling of the obtained haploid plants is achieved with colchicine (the compound used to double the number of chromosomes in cells) or other mutagens. Obtained plants are 100% homozygous. Anther culture is also used in hybrid breeding. Haploidisation and diploidisation methods have been preferred for many years since it was discovered since it not only shortens the time in breeding studies but also can obtain fully homozygous plants in incompatible species in a short time (Guha and Maheshwari, 1966; Kristiansen and Andersen, 1993; Sopory and Munshi, 1996; Ercan and Şensoy, 2012). It has been reported that many breeding studies are carried out with the anther culture method, but the success in techniques and methods is different from species to species and in varieties, and even it does not provide the same success in other studies (Irikova et al., 2011). In this study, the definition of anther culture, its application and the factors affecting success, the research made with the anther culture method in the world and in our country, and the developments in recent years are discussed.

2. Anther Culture Studies

It is reported that the first research in tissue culture in plants started with Schwann and Schleiden's theory of totipotency in 1838 (Pierik, 1987). *Solanaceae* was described by Guha and Maheswary (1964) 1964 as the first haploid plant in its family with anther culture technique. He described the first haploid plant in his family. Obtaining haploid plants by anther culture method in the *Capsicum* genus and especially in pepper species; Wang et al. (1973), Saccardo and Devreux (1974), Harn et al. (1975),

Sibi et al. (1979), Dumas de Vaulx et al. (1981), Morrison et al. (1986), the first studies started with researchers, and the first studies in our country were Abak (1983), Tıpırdamaz and Ellialtıoğlu (1998), Abak et al., (1998), Özkum et al., (2001) and Çomlekcioglu et al., (2001). It was initiated by the researchers and contributed to the development of the method.

In a study, different temperature applications (Dumas de Vaulx et al., 1981) were applied to increase embryo formation, studies such as different nutrient media and pre-applications were carried out to obtain haploid plants by anther culture method in local peppers of Turkey origin, and it was reported that successful results were obtained (Abak, 1983). Anther culture method was used to obtain in vitro haploid plants in Kahramanmaraş local red pepper genotypes. Mononuclear pollen stage anthers from flower buds taken from donor plants were transferred to MS medium, at the same time, they were cultured by leaving different amounts of hormonal contents in the nutrient medium. Three different pre-temperatures (4°C, 29°C, and 35°C in the dark) were applied seven days apart. In the study, 37 different applications (pre-temperature applications with different auxin-cytokinin, activated charcoal, and $AgNO_3$ nutrient media) were applied to the anthers; 9750 units of anthers were used and it was reported that while callus formed in some nutrient media, no callus was formed in others (Çağlar et al., 2004). In the study conducted by Sayılır and Özzambak (2005) in 6 pepper varieties; the anther culture method was applied by forming 3 groups as pointed, stuffed, and Banana type. Appropriately sized flower buds (5-6 mm in length) were inoculated into 6 different media with combinations of activated charcoal, carrot extract, 4mg/l NAA+0,1mg/l BA in MS and NN nutrient media. The most successful result was the combination of MS+4 mg/l NAA+ 0,1 mg/l BA, and the carliston variety, N+4mg/l It has been reported that the nutrient medium consisting of NAA+0,1mg/lBA +0,1% activated charcoal+200ml carrot extract was not successful. In another study, somatic embryogenesis, organogenesis, and clonal production were determined in five different pepper varieties (Tunceli, Kıl sweet, Irmak F1, İnce pointed, and Demre) research has been done on it. In clonal production; knuckle, stem tip, leaf and leaf Leaf explants were used in the internodes for the stem parts and the zygotic embryo, in somatic embryogenesis and organogenesis. As a result of the study; Embryogenic callus and proembryo formation at 4 mg/l 2,4-D in somatic embryogenesis Irmak in F1; It was found that 6±1,53 shoots developed at the stem end in 25 mg/l BAP medium in the Bristle sweet pepper cultivar, and in organogenesis studies, Irmak F1, Bristle sweet and fine pointed. It has been determined that root and shoot develop in 4–5 mg/l 2,4-D medium in the knuckle and leaf parts of cultivars (Dalar, 2008).

Lantos et al. (2009) investigated the response of three Hungarian and three Spanish pepper varieties on pepper genotypes by using the microspore culture technique. As a result, it was stated that anthers with 80% seedless and 20% binucleated microspores were successful and gave the highest frequency of successful microsporous cultures. Isolation in different pepper genotypes used in the experiment. It has been reported that there are differences in the transformation from embryo formation to plant in pepper microspore culture. In another study, anther culture method was used to determine the effect of

genotype on 11 different peppers (2 long green, 4 long, 2 capia, 1 red pepper, 2 bell pepper) (Ercan and Şensoy, 2011). After collecting flower buds from genotypes, anthers were placed in MS (Murashige-Skoog) media in prepared petri dishes. The number of anthers used in 11 genotypes is 2398; embryo formation is 44 and plant yield is 12; It was reported that there was no embryo formation in long green pepper type and bell pepper type, and androgenic response was more successful in long pepper type and bell pepper type compared to other pepper genotypes. Weekly flower buds were collected from donor plants grown in winter (November and May) and summer (April and December) varieties of pepper (Kekova and Sera Demre 8) using anther culture method. The results obtained by placing the anthers on the nutrient media were compared. In the study; the success rate is high in young plants, and the success increases when suitable varieties are used according to the season; As the plant age increases, the rate of embryo formation decreases. reported (Ercan et al., 2011). Taskin et al. (2011) tried four different culture media in order to increase the embryo formation rate and plant transformation rate in pepper genotypes that were inbred three times (low temperature, moderately tolerant and sensitive) using the anther culture method. It was reported that embryo formation was best in the 269 genotype, in April and May, the nutrient media numbered 3 and 4 were more successful than the other nutrient mediums, and there was a plant transformation in the hormone-free MS medium.

Lantos et al. (2012) inducing capacities, cell divisions, and microspore formation in anthers of sweet pepper F1 hybrid (*Capsicum annuum* L) genotypes in which the microspore culture technique was applied was investigated. Embryo-like in experiment are formations; It has been reported that there are 1,5 plants with an average of 48,1 embryo-like formations in 12 genotypes, and there are also abnormal shoot formations in embryo-like formations. Özsan (2014) in a study he carried out, aimed to determine the haploid plant acquisition rate of genotypes by using pepper genotypes at 4 different genetic stages to obtain haploid plants by applying anther and microspore cultures. In the study, after the flower buds were classified, the anthers were transferred to MS media. The embryo formation rate was 1507% in anther culture and 13,89% in microspore culture has been reported. Alremi et al. (2014) used anther culture method to obtain haploid plants in 3 different pepper genotypes and 1 pepper variety (Alfajer), while 16 different nutrient media combinations and genotype effects were also investigated.

Eight different combinations of DDV media and 8 different MS nutrient media combinations were used. Alfajer in the Study and B line pepper genotypes have been reported to have better results than C series media containing Kinetin + 2,4-D. MS medium was used with anther culture method to increase the embryo formation rate in 73 pepper genotypes and 11 standard varieties collected from different locations in Turkey (Kaplan, 2012). They reported that the anther number used in these 84 pepper genotypes was transferred to 7343 media, there were 70 calli, there was no embryo or shoot development in pepper anther culture application, and the development of androgenesis stimulation protocols was important and necessary for studies in this direction.

Durna (2016) in his study, used anther culture method in 10 peppers to obtain embryo formation and haploid plants; It was aimed to determine the effects of preliminary studies, nutrient media and incubation conditions. Anthers that used DDV nutrient media kept flower buds at +4 °C for 24 hours before transferring to the medium; This had a positive effect on embryoid formation. In the study results genotype, pre-treatments, nutrient medium and incubation applications increase success; It was determined that 1109 embryoids, 210 plantlets, and 27 DH plants were formed and one of the important results of the study was that embryo formation was more successful from F1-stage genotypes rather than F2-stage genotypes. In the study conducted by Çelik (2016) haploid plants were obtained by using anther culture method in 3 pepper and 1 Tokat local pepper genotypes; genotype, nutrient environment, stress, and incubation stages were also examined. Two environments, DDV and MS, were applied; while the highest embryo formation was in Tokat local pepper genotype, embryo formation was less in other genotypes; while the embryo formation rate in MS medium was 3,9%, it was reported that the embryo formation rate was 9,33% in DDV medium. In a study by Erim (2019) the shed microspore method was used in commercial cultivars of 3 different pepper genotypes. In the experiment, the lengths of the anthers, anthocyanin levels, and development stages of microspores in anthers, embryo formation and plant transformation rate, and embryo formation of liquid nutrient medium in shed microspore method potential have been explored. In the results of working; shed microspore culture method with 3 different 443 embryos were formed in the genotype and 85 were found to be normal. It has been reported that the highest rate of embryo formation and haploid plantlets are in bell pepper (Benino F1), the best response with normal embryo formation rate is capia pepper, but the chili pepper genotype responds slightly in this method.

Özsoy (2019) using the anther culture method, supplemented nutrients at different rates in different (DDWX and MS) nutrient media in 4 hybrid pepper cultivars, and the effect on the embryo formation rate by applying it at low temperature conditions at the same time has researched. Before the anthers were transferred to the media, flower buds were kept at 4 to 10ºC for 24 to 48 hours. As a result of the applications, DDWX and MS environments were more successful than B5 environments; highest in DDWX and MS environments. It was determined that there was embryo formation and haploid production by transformation into a plant was in the Wish F1 genotype. Pre-applications, nutrient media in anther culture ıt has been reported that it affects performance and is an important factor in cold shock application. Hülül (2019) in a study he carried out, in order to obtain haploid plants in 5 pepper genotypes [Fine pointed (B4), capia (B16), bell pepper (B90), cubanelle (B85) and banana type (B54)] by anther culture method used the environment; in embryo formation, genotype, sugar content and the effect of plant growth regulators were also investigated. It was reported that the highest embryo formation was in the three nose (B85) genotypes, but also changed the success rate in genotype, sugar content and plant growth regulators. Shimira et al. (2019) had a low response to androgenesis with the Pili-pili pepper variety (*Capsicum chinense*), originally from Rwanda, as a control. The response of the A11 genotype and the highly responsive Inan 3363 genotypes to anther

culture was investigated. MS environment was used in the study; from the control groups, believe 19,4 embryos/100 anthers were obtained from 3363 cultivars, and 4,46 embryos/100 anthers from A111 genotype; It was reported that embryos could not be obtained in Rwanda Pili-Pili pepper cultivar.

In another study, MS (Murashige and Murashige and Skoog) nutrient medium was used to measure the response of 23 different pepper genotypes to anther culture (Atasoy, 2020). The number of embryos in 100 anthers used in the study. ranges from 0,83 to 44,44; It was determined that the highest performance was in the FT-509 genotype, the lowest performance was in the FT-1178 genotype with 0,83 embryos/100 live anthers and all embryos turned into plants. Ceyhan and Aktaş (2020) used anther culture method to obtain haploid plants from 4 different varieties (Üçburun, Buket, B22 and B23) consisting of cubanelle and stuffed peppers, and in order to increase the success of this method, genotype and nutrient ın this study, the response of antioxidants (zinc, salicylic acid and vitamin C to nutritional media) applied in varying amounts was investigated. MS nutrient medium was used and anthers were cultured after 24 hours of cold application at 4°C after collecting flower buds. As a result of the study, the highest embryo formation was in the cubanelle varieties, while there was no embryo formation in the Buket variety; Transformation into plants has been reported in all cultivars with embryo formation. In the studies of Grozeva et al., (2020) anther culture method was used to obtain haploid plants in a four-parent pepper genotype. In the study, morphological features of genotypes, vitamin C, dry matter content, total Quality properties such as polyphenols and antioxidants were also investigated. There was also a significant difference in fruit morphology, quality and production between the obtained DH lines and parent genotypes has been reported.

In this study, it was aimed to determine the morphological and molecular characterization and the possibilities of obtaining double haploid (DH) lines in Gaziantep local pepper (GB) genotypes. In the study, 96 pepper genotypes, 81 different GB genotypes and 15 standard varieties, collected from the region were used. In the dendograms obtained as a result of morphological and molecular analyzes, 15 groups were formed; In these groups, MS medium was applied in the anther culture method in the spring and autumn periods. It has been determined that anther culture made in the spring period is more successful than the anther culture made in the autumn period. While the genotypes in the 9th group, including the 10th and 12th groups, were the most successful in the autumn period, although the genotypes in the same group were not as successful as in the spring period, it was observed that they were relatively successful in the spring period. In anther culture studies, haploid and dihaploid plants and fruits were obtained (Tatar, 2022).

3. Conclusion

Pepper is one of the important types of vegetables worldwide. In our country, there are populations that have taken local names in most regions. However, although these local varieties are preferred in terms of flavor and aroma, they do not provide the desired performance in productivity and disease resistance.

Increasing seed yield and production, which is one of the most important factors of plant production, is also very important in obtaining durable, low-cost and competitive products. F1 hybrid seeds are obtained as a result of crossing two different parent plants. The parents used in crossbreeding consist of homogeneous lines. Therefore, F1 hybrid cultivars are being developed, especially by utilizing local genetic resources. Studies have reported that pepper is a highly self-pollinating plant, but the foreign pollination rate also increases depending on the factors affecting pollination.

In pepper breeding, homozygosity level is increased in each generation, and at the end of inbreeding, nearly 100% homozygous lines are obtained. However, developing varieties with classical breeding studies takes many years and requires a lot of time and effort. Today, tissue culture techniques are used to shorten this period and obtain more guaranteed results. Anther culture technique is one of the widely used techniques for obtaining haploid plants. The advantage of this technique over other in vitro haploid plant production techniques; the presence of thousands of microspores in an anther and the ability to obtain a large number of haploid plants from an anther.

It is known that the anther culture technique gives successful results in many species of the *Solanaceae* family. Dihaploid line was obtained for the first time in pepper, which is in the *Solanaceae* family, by anther culture method, and other studies examining the effects of many factors and developing more successful protocols were carried out. In recent years, successful results have been obtained in pepper anther culture; These are the important factors affecting success; These are the factors originating from the plant from which the anthers are taken (genotype and the growing conditions of the donor plant) and the anther culture technique (development period of the anthers, pre-applications, composition and structure of the nutrient medium and incubation conditions). Researchers have made different experiments for many years, especially on the nutrient medium and genotypes. Success in anther culture varies according to genotypes and each genotype reacts. It was observed that the nutrient media also differed.

Studies have shown that genotype, nutrient medium, studies continued on the effects of growth regulators, different substances added to the nutrient medium (such as activated charcoal, silver nitrate), different pre-treatments, growing conditions of donor plants, and different anther uptake times. Studies in this direction aimed to increase the number of haploid embryos obtained by anther culture and to increase the efficiency of anther culture method in pepper breeding. Anther culture method is successful in breeding studies and varieties are developed with this method.

Conflict of Interest Statement

The article author declares that there is no conflict of interest.

Contribution Rate Statement Summary of Researchers

The author declares that she has contributed 100% to the article.

References

Abak K. Research on obtaining haploid plants by anther culture in pepper (*Capsicum annuum* L.). Ankara University Faculty of Agriculture Yearbook. Volume: 33, Separate Edition From Fascicules 1983; 1-4(1-2-3-4): 155-163.

Abak K., Comlekcioglu N., Buyukalaca S., Sari N. Use of stomatal characteristics to estimate ploidy level of haploid and dihaploid pepper plants. In: Xth EUCARPIA Meeting on Genetics and Breeding of *Capsicum* and Eggplant, Avignon France 1998; 179–182.

Abak K. Utilizing anther culture in pepper breeding. Tübitak Plant Breeding Symposium Abstracts, October 1986; 15-17(15-17): İzmir 64.

Ari E., Yildirim T., Mutlu N., Buyukalaca S., Gokmen U., Akman E. Comparison of different androgenesis protocols for doubled haploid plant production in ornamental pepper (*Capsicum annuum* L.). Turkish Journal of Biology 2016; 40(40): 944-954.

Alremi F., Taşkın H., Sönmez K., Büyükalaca S., Ellialtıoğlu Ş. The effects of genotype and nutrient medium on anther culture in pepper (*Capsicum Annuum* L.). Turkish Journal of Agriculture and Natural Sciences 2014;1(2): 108–116.

Andrews J. Peppers, the domesticated *capsicum*. University of Texas Press, Austin, Texas, 1985

Atasoy D. Determination of anther culture performance of some pepper (*Capsicum annuum* L.) breeding genotypes (master's thesis). Çukurova University, Graduate School of Natural and Applied Sciences, Department of Horticulture, Adana 2020.

Ceyhan AP., Aktaş H. Development of dihaploid three cape and stuffed pepper lines with anther culture technique. Eurasian J Bio Chem Sci, 2020; 3(Appendix 1): 199-205,

Çağlar G., Aras V., Bayram A. In vitro haploid embryo stimulation by androgenesis in dried red peppers. Journal of Akdeniz University Faculty of Agriculture 2004; 17(1): 87-94.

Çelik ME. Obtaining dihaploid lines by ın vitro androgenesis method in three nose pepper genotypes (master's thesis). Gaziosmanpaşa University, Institute of Science and Technology, Department of Horticulture, Tokat 2016.

Çömlekçioğlu N. Breeding of southeast anatolian pepper populations by dihaploidization method and comparison with traditional methods (doctoral dissertation). C.U. Science Science Inst. Adana 2001.

Çömlekçioğlu N., Büyükalaca S., Abak K. Effect of silver nitrate on haploid embryo ınduction by anther culture in pepper (*Capsicum annuum* L.). XI. th. Eucarpia Meeting on Genetics and Breeding of *Capsicum* and Eggplant April 9-13 Antalya 2001.

Dalar A. Microproduction of pepper (*Capsicum annum* L.) plant varieties by different tissue culture methods (master's thesis). Yuzuncu Yıl University, Institute of Science, Department of Biology, Van 2008.

Dumas R., Chambonnet D., Pochard E. In vitro culture of pepper (*Capsicum annuum* L.) anthers: high rate plant production from different genotypes by +35 0C treatments. Agronomie 1981; 1(10): 859-864.

Durna P. The effect of ın vitro androgenesis applications on dihaploid line development in some pepper genotypes and morphological characterization of dihaploid lines (PhD thesis). Gaziosmanpaşa University Institute of Science and Technology, Tokat 2016.

Ellialtıoğlu Ş., Başay S., Kuşvuran Ş. Comparison of ın vitro and ın vivo colchicine applications used for doubling of haploid eggplants obtained from anther culture. VI. Vegetable Agriculture Symposium 2006; September 19-22, Kahramanmaraş 386-390.

Ellialtıoğlu S., Tipırdamaz R. The effect of cold applications and activated charcoal on the change in abscisic acid amount during anther culture in pepper (*Capsicum annuum* L.). Journal of Akdeniz University Faculty of Agriculture 2002; 15(1): 9-18.

Ercan N., Sensoy FA. Determination of the optimum microspore development stage and optimum culture medium in asparagus (Asparagus officinalis var. altilis L.) for anther culture. Acta Horticulturae 2012; 961, 153-157.

Ercan N., Sensoy FA., Sensoy AS. Inftuence of growing season and donor plant age on anther culture response of some pepper cultivars (*Capsicum annuum* L.), Scientia HorticuIiurae 2011b; 110 (2006): 16-20.

Erim FB. Application of shed microspore culture technique on different pepper (*Capsicum Annuum* L.) genotypes and determination of microspore development stages (master's thesis). Akdeniz University, Institute of Science, Department of Horticulture, Antalya 2019.

Grozeva S., Tringovska I., Nankar AN., Todorova, V., Kostova, D. Assessment of fruit quality and fruit morphology in androgenic pepper lines (*Capsicum annuum* L.). Crop Breed Genet Genome 2020; 2 (1): e200005.

Guha S., Maheshwari SC. Cell division and differentiation of embryos in the pollen grains of datura in vitro. Nature (Lond.) 1966; 212, 97-98.

Harn C., Kim MZ., Choi KT., Lee YI. Production of haploid callus and embryoid from the cultured anther of *Capsicum annuum*. Sabrao 1975; 7: 71–77.

Heiser CBR. Peppers, in evolution of crop plants. Longman Science 1976; 265-268.

Hülül M. The effect of plant growth regulators and sucrose doses on androgenic embryo formation in some pepper genotypes (master's thesis). Tokat Gaziosmanpaşa University, Institute of Science and Technology, Department of Horticulture, Tokat 2019.

Irikova T., Grozeva S., Rodeva V., Anther culture in pepper (*Capsicum annuum* L.) in vitro. Acta Physiologiae Plantarum 2011a; 33, 1559-1570.

Kaplan FN. Production of dihaploid lines from pepper (*Capsicum Annuum* L.) breeding materials (master's thesis). Pamukkale University Institute of Science and Technology, Department of Biology, Denizli 2012.

Khush GS., Virmani SS. Somatic embryogenesis in cultured unfertilized ovules of *Cucurbita moschata*. Journal of the Japanese Society for Horticultural Science 1996; 57(1): 34-42.

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

Lantos C., Juhasz AG., Somogyi G., Ötvös VP., Milhaly R., Kristo Z., Somongyi N., Pauk J. Improvement of isolated microspore culture of pepper (*Capsicum Annuum* L.) via co-culture with ovary tissues of pepper or wheat. Plant Cell Tiss Organ Cult 2009; 97: 285–293.

Lantos C., Juhasz AG., Vagi P., Mihaly R., Pauk, ZKJ. Androgenesis induction in microspore culture of sweet pepper (*Capsicum annuum* L.). Plant Biotechnol Rep 2012; 6(123): 132.

Morrison RA., Koning RE., Evans DA. Anther culture of intersspecific hybrid of *Capsicum.* J. Plant Physiol 1986; 126:1-9.

Murashige T., Skoog F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia Plantarum 1962; 15 (3): 473-497.

Olszewska D., Kisiala A., Niklas- Nowak A., Nowaczyk P. Study of in vitro anther culture in selected genotypes of genus *Capsicum*. Turkish Journal of Biology 2014; 38, 118-124.

Ozsan T., Onus AN. Can in vitro pepper (*Capsicum annuum* L.) anther culture be affected by B vitamins? International Journal of Biotechnology 2017; 20(1): 1-13.

Özkum D., Tipirdamaz R., Ellialtıoğlu S. The relationship between the endogenous abscisic acid content of anthers and in vitro androgenesis in pepper (*Capsicum annuum* L.). Acta Hortic 2001; 560, 327–329.

Özsan Ö. Effects of plant use at different genetic progression levels on haploid plant obtainment in pepper (*Capsicum Annuum* L.) Anther and Microspore Culture (master's thesis). Akdeniz University, Institute of Science, Department of Horticulture, Antalya 2014.

Özsoy B. The effects of genotype, nutrient environment and stress applications on androgenesis in pepper (master's thesis). Tokat Gaziosmanpaşa University, Institute of Science and Technology, Department of Horticulture, Tokat 2019.

Parra-Vega V., Seguí-Simarro JM. Anther culture in pepper (*Capsicum annuum* L.). Methods in Molecular Biology (pp. 1359). New York, NY 2016.

Pierik RLM. In vitro culture of higher plants. Martinus Nijhoff Publishers. Dordrecht, The Netherlands 1987; 344.

Popova T., Grozeva S., Todorova V., Stankova G., Anachkov N., Rodeva V. Effects of low temperature, genotype and culture media on in vitro androgenic answer of pepper (*Capsicum annuum* L). Acta Physiologiae Plantarum 2016; 38, 273.

Saccardo F., Devreux M. In vitro production of plantlets from anther culture of *Capsicum annuum* L. Proc. of Eucarpia: Genetics and Breeding of *Capsicum*, Budapest 1974; 45-49.

Sayılır A., Özzambak E. A research on the effects of appropriate bud size and nutrient media contents on embryo yield in pepper anther culture. Ege Univ. Agriculture. Fac. Journal 2005; 42(3): 1-11.

Shimira F., Yıldız S., Baktemur G., Keleş D., Aydın MZ., Büyükalaca S., Taşkın H. Investigation of androgenesis capacity of rwandan pili-pili variety (*Capsicum chinense* L.) in Turkey. Yüzüncü Yıl University Journal of Science Institute 2019; 24(3): 170-175.

Sopory SK., Munshi M. Anther culture in vitro haploid production in higher plants. Current Plant Science and Biotechnology in Agriculture 1996; 23.

Sibi M., Dumas De Vaulx R., Chambonnet D. Obtention de plantes haploides par androgene in vitro chez le piment (*Capsicum annuum* L.). Ann Amelior. Plantes 1979; 29: 583-606.

Taşkın H., Büyükalaca S., Keleş D., Ekbic E. Induction of microspore-derived embryos by anther culture in selected pepper genotypes. African Journal of Biotechnology 2011; 10(75): 17116-17121.

Tatar M. Morphological and molecular characterization of Gaziantep local pepper genotypes and the possibilities of obtaining double haploid (DH) lines (PhD thesis). Yuzuncu Yıl University, Institute of Science, Department of Horticulture, Van 2022.

Tıpırdamaz R., Ellialtıoğlu Ş. The effect of cold applications and activated charcoal on the change in abscisic acid content during anther culture in pepper (*Capsicum annuum* L.). Journal of Akdeniz University Faculty of Agriculture 2002; 15(1): 9-18.

Wang YY., Sun CS., Wang CC., Chien NJ. The induction of pollen plantlets of Triticale and *Capsicum annuum* from anther culture. sci. Sin 1973; 16: 147-151.