Selcuk Journal of Agriculture and Food Sciences

http://sjafs.selcuk.edu.tr/sjafs/index

Research Article

SJAFS

(2022) 36 (2), 253-259

e-ISSN: 2458-8377

DOI:10.15316/SJAFS.2022.033

Doublehaploidization Efficiency of Selected Pepper Genotypes Via in Vitro Anther Culture

Münevver İLHAN[1,*], Ertan Sait KURTAR[1]

Selçuk University, Faculty of Agriculture, Department of Horticulture, Konya, Türkiye

ARTICLE INFO

Article history:
Received date: 03.03.2022
Accepted date: 04.07.2022

Keywords:
Anther culture
Pepper
Doublehaploidization

ABSTRACT

This study was carried out to determine the effects of genotype and media on haploid plant formation of 12 pepper genotypes (*Capsicum annuum* L.) via *in vitro* anther culture. The buds were cultured in 2 different media (O1 and O2) and, the results revealed that the media had effects on the development of anthers, embryo and plantlet initiation. When the anther developmental status is examined, the lowest value was obtained from the SU-31 genotype with 2.47%, while cv. Flinta F1 produced the highest rate with 12.79%. The highest growth rates were obtained from the O2 medium, and the cv. Dolphin variety produced a remarkable result with 25.0% in this medium. The cultivar Dolphin was the favorable genotypes with 8 embryos and 5 plants, while no embryo and plant were obtained from cv. Flinta F1 and SU-34. A total of 2868 anthers were cultured, 195 anthers were enlarged and the growth rate was 6.8%. Finally, 37 embryos and 18 plants were obtained and the frequencies were 1.29% and 0.63%, respectively. As a result of stomatal observations, 13 plants were detected haploid and the others were double haploid.

1. Introduction

Pepper (*Capsicum annuum* L.), whose origin is Central and South America, is a type of vegetable grown economically in Europe since the 15th century and in Turkey since the 16th century. The genus Capsicum includes about 30 species, and *C.annuum*, *C. baccatum*, *C. pubescens*, *C. frutescens* and *C. chinense* are important economically cultivated species. All-natural pepper populations are diploid (2n), with chromosome numbers of 2n=24. Pepper is one of the most consumed vegetable worldwide. The versatile pepper is grown in the open field and protected cultivation at all of the year. It is used in many different forms such as raw, cooked, dried, canned, powdered and chili powder, pepper paste and sauce. World pepper production is 62.7 million tons in 5.33 million hectare areas and the world's top pepper-producing countries are China (19 million tons), Mexico (3.23 million tons), Turkey (2.62 million tons), Indonesia (2.58 million tons) (FAO, 2019). Turkey's pepper production was 2 63 million tons in 77 800 ha, of which 1 291 091 tons of capia pepper (paste), 389 957 tons of bell pepper, 838 890 tons of long green pepper, 116 967 tons of charleston pepper (TUIK, 2020).

Pepper has high economic importance in Turkey and the world; is frequently preferred by breeders in breeding programs. On the other hand, although the seed production of F1 hybrid cultivars used in vegetable growing is more difficult and costly than standard varieties, the reason why F1 hybrid varieties are preferred is that these varieties are more productive than standard varieties, they have wider adaptability and are also resistant to various diseases. Superior varieties can be obtained more quickly with F1 hybrid power (heterosis) breeding. Pepper is known as a highly self-pollinating plant, and the open pollination rate can reach up to 79%.

In classical pepper breeding, it takes a long time to reach a high homozygosity rate in parent lines, but 100% purity cannot be achieved in any way. In addition, the goals that can be reached are limited. Using *in vitro* techniques, 100% homozygous lines can be obtained in a shorter time. Among the *in vitro* techniques the haploid plant production technique, which we can be successful in some species, can have important advantages for breeders. In doblehaploidization (DH) technique, which is one of the *in vitro* techniques used in plant breeding, the aim is to obtain haploid (n) plants originating from the male (anther-pollen culture) and female (ovule-ovary culture) germ cells or parthenogenesis (irradiated pollen

* Corresponding author email: *ertansaitkurtar@hotmail.com*
This article was produced from master thesis.

technique). It is possible to 100% pure (homozygous) doublehaploid plants (2n) by doubling their chromosome numbers with various antimitotic agents. Plants with the number of chromosomes in their somatic cells equal to the number of chromosomes in the gamete cells of the plant species to which they belong are called haploid plants. Since haploid plants contain only one set of chromosomes, they allow recessive mutations to be revealed. To obtain 100% homozygous pure and fertile lines from haploid plants, the chromosome numbers must be doubled (Ellialtıoğlu et al., 2001).

The first doublehaploid pepper lines were obtained from anther culture by Wang et al. (1973) and George and Narayanaswamy (1973). Afterward, studies focused on developing more successful protocols (Dumas de Vaulx et al., 1981; Dumas de Vaulx, 1989). Studies on androgenesis in pepper intensified at the end of the 20th century and studies are continuing intensively today (Rodeva and Cholakov, 2006; Kim et al., 2008; Lantos et al., 2009; Irikova et al. et al., 2011a; Barroso et al., 2015; Çömlekçioğlu et al., 1999; Çömlekcioglu et al., 2001; Ellialtıoğlu et al., 2001; Çiner and Tipirdamaz, 2002; Buyukalaca et al., 2004; Taskin et al., 2011; Al Remi et al., 2014; Arı et al., 2016; Durna, 2016).

Although successful results have been reported in pepper by anther culture in recent years, the number of studies is not intensive and there is still no general protocol reported in all genotypes (Irikova et al., 2011b). The genotype effect, which is one of the most important factors affecting the success in anther culture, still causes the desired response or very low success in many pepper genotypes. In addition, if doublehaploid lines are thought to be used in hybrid pepper cultivar breeding, as many haploid lines as possible are needed. Considering the reasons mentioned above, the level of success achieved in anther culture in pepper today should not be considered sufficient. In this study, which started with this approach, the effects of the medium on *in vitro* androgenetic response of some pepper genotypes were investigated.

2. Materials and Methods

2.1. Donor Plants

Eight pepper genotypes (SÜ-29, SÜ-30, SÜ-31, SÜ-32, SÜ-33, SÜ-34, SÜ-35 and SÜ-36) have been collected from our gene pool due to some agronomic characteristics at the S3 stage and 4 commercial pepper cultivars (Klasman F1, Flinta F1, Doru 16 and Dolphin) were used as plant materials. Among the genotypes, "SÜ-29" is Urfa pepper, "SÜ-30", "SÜ-31" and cv. "Klasman F1" are Charleston type, "SÜ-32" and cv. "Flinta F1" are sweet-long type, "SÜ-33" is bitter-long type, "SÜ-34" and cv. "Doru 16" are green-bell type, "SÜ-35" is red-bell type, "SÜ-36" is yellow-bell, and cv. "Dolphin" is capia type.

2.2. Cultivation of Donor Plants

The seeds were sown in plastic boxes containing peat under greenhouse conditions. Seedlings at the stage of 3-4 leaves were planted in greenhouse conditions at 0.7x0.6 m distances, and 20 plants were used for each genotype. After planting, sap water was applied with the drip irrigation system placed in the greenhouse.

The plants were grown healthy by fertilizing twice a week during the plant development period according to the soil analysis. Cultural practices such as weeding, hoeing, and disease and pest management were applied properly and timely. Fruits were discarded continuously, and it was ensured that they produced healthy anthers continuously during the study period.

2.3. Determination of Appropriate Microspore Stage in Anthers

The uninucleate stage in pollen development was determined via the crush-preparation method at the flowering stage. For this purpose, flowers at different developmental stages were collected and the anthers were excised from flowers and were placed on a slide, 1-2 drops of 1% acetocarmine solution were dripped and the anthers were crushed. The samples were examined under the light microscope at 40 x 10 magnification. In this way, anthers containing microspores between the late uninucleate and early binucleate stages were cultured. The size of the buds containing the anthers in this stage was determined, thus the buds at the 3rd and 4th stages were suitable, where the purple color (anthocyanin) was at the tip or up to 1/3 of the anther (Figure 1). Buds at this stage were used thoroughly in the study.

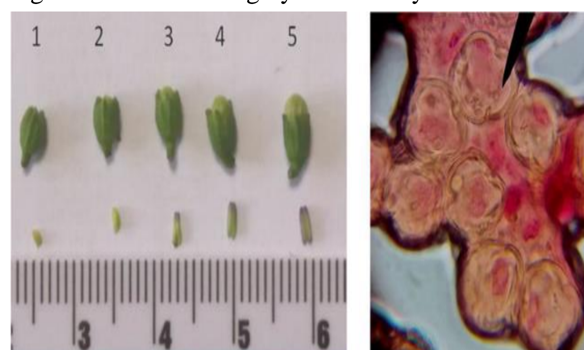

Figure 1
Determination of suitable bud size (left), uninucleate microspores (Right – 400x)

2.4. The Media Composition

The protocols of Taskin et al. (2011) and Arı et al. (2016) were implemented partially modified (Table 1). The media was autoclaved at 121 C°, 1.1 atm for 15 min and poured with an automatic pipette, with 5 ml in each 6 cm diameter sterile Petri dishes under sterile conditions.

Table 1
Media composition

Media	Composition
O1	MS+0.5 mg/L BAP+4 mg/L NAA+15 mg/L silver nitrate+0.25% activated carbon+30 g/L sucrose+ 8 g/L agar, pH 5.8 (Taşkın et al., 2011).
O2	B5 + 0.5 mg/L BAP + 4 mg/L NAA, + 15 mg/L silver nitrate + 0.25% activated carbon + 30 g/L sucrose + pH 5.8 + 8 g/L agar (Arı et al., 2016)

2.5. Surface Sterilization and Anther Preparation

Buds of appropriate size were collected early in the morning and subjected to surface sterilization for 15 min in a 15% commercial bleach solution. Then the buds were rinsed with sterile distilled water 3 times for 5 min and excessive water was removed with sterile blotting papers. Sterilized buds were carefully opened and filament-free anthers were placed in 6 cm diameter sterile plastic Petri dishes filled with nutrient medium. After the sewing process was completed, the edges of the dishes were closed with stretch films. The anthers were incubated at 35 °C in the dark for 2 days, then were transferred 25 °C and 16/8-h photoperiod.

2.6. Obtaining Plants and Acclimatization

Plantlets that started to appear approximately 30 to 60 days after culture and formed cotyledons were taken into germination medium containing MS+0.01 mg/IAA+30 g/L sucrose+8 g/L agar. Plantlets that reached sufficient size were grown in 250 ml jars containing the same medium to complete their development. Plant numbers were multiplied by applying 2-3 micro cuttings for preventing plant losses.

In the process of acclimatization to field conditions, firstly, sterilized peat was filled into plastic cups, and the roots of the plants were removed from the culture medium, and cleaned under tap water, and were planted in 150 ml plastic cups. Before planting, the curled, darkened, and overgrown roots of the plants were discarded, and 1% fungicide solution was added to the sap water to prevent fungal contamination during the acclimatization process. Then, the plants were covered with a plastic bag, and 2 small holes were opened in the bag every two days, and the plants were acclimatized to the field conditions for 2 weeks (Figure 2).

Figure 2
Acclimatization process

2.7. Determination of Ploidy Status and Dihaploidization Process

Ploidy determination was realized by stomatal observations (the number of stomata in mm^2, the stomata width and length, and the number of chloroplasts in guard cells), which are routinely used in pepper. For this purpose, the lower epidermal parts of the leaves taken from the acclimatized plants were placed on a slide and 1-2 drops of 1% silver nitrate solution were dropped and examined under a microscope. Plants determined to be haploid were planted and grown in 2.5-liter pots filled with 2 peat:1 perlite mixture. *In vivo* colchicine applications were realized at 0.5% concentration for 12 hours. Colchicine solution was applied to axillary buds with immersing method. Thus, the excessive leaves were pruned and a piece of cotton was placed onto the buds and wrapped by aluminum foil. Finally, 1-2 ml of colchicine solution was injected into cotton. The leaves of colchicine applied shoots were examined again by stomatal observations. Colchicine applications were continued to the haploid plants until chromosome doubling was achieved (Figure 3).

Figure 3
Application of colchicine to the haploid plants

In addition, visually ploidy differences were observed in haploid and doublehaploid plants planted in the greenhouse. Although no measurements were made in these observations, it was determined that the development of the vegetative organs of double haploid plants and haploid plants was different, the leaves and flowers of haploid plants were smaller, the plant height was shorter and they developed more slowly.

During the study, the number of anthers cultured (NA), the number of anthers developed callus (NC), the number of developing anthers (NDA), embryo number (EN), embryo frequency (% EF), plant number (PN), plant frequency (% PF) and conversion rate to plant (% CRP) were determined. Since an equal number of materials could not be used for each application statistical analysis could not be realized, only the averages were presented.

3. Results and Discussion

During the experiment, a total of 2260 anthers were cultured on O1 and O2 media. The highest anther development rates were obtained from Flinta F1 (15.94%) and cv. Klasman F1 (15.79%), followed by cv. Doru 16 (8.33%). None of the SÜ-31 anthers developed in the O1 medium. In the O2 medium, while anther development was 25.00% in cv. Dolphin, and 16.67% in cv. Doru 16 and SÜ-32, the SÜ-36 genotype had the lowest value (6.94%). Anther development was obtained in O1 and O2 media from all genotypes except SÜ-31 (Table 2).

Table 2
The number of anthers cultured (NA), the number of anthers developed callus (NC), number of developing anthers (NDA)

Genotypes	Mediums	NA	NC	NDA
KLASMAN F1	O1	114	18	15.79
	O2	48	6	12.50
	∑	162	24	14.81
FLINTA F1	O1	138	22	15.94
	O2	66	11	16.66
	∑	204	33	16.17
DORU16	O1	60	5	8.33
	O2	54	9	16.67
	∑	114	14	1.28
DOLPHIN	O1	216	6	2.78
	02	84	21	25.00
	∑	370	27	7.29
SÜ 29	O1	102	8	7.84
	O2	114	17	14.91
	∑	216	25	11.57
SÜ 30	O1	60	1	1.67
	O2	84	6	7.14
	∑	144	7	4.86
SÜ 31	O1	72	0	0.00
	02	48	4	8.33
	∑	120	4	3.33
SÜ 32	O1	192	4	2.08
	O2	66	11	16.67
	∑	258	15	5.81
SÜ 33	O1	96	7	7.29
	O2	72	6	8.33
	∑	168	13	7.73
SÜ 34	O1	72	2	2.78
	O2	48	5	10.42
	∑	120	7	5.83
SÜ 35	O1	102	3	2.94
	O2	72	9	12.5
	∑	174	12	6.89
SÜ 36	O1	138	9	6.52
	O2	72	5	6.94
	∑	210	14	6.66
	General	2260	195	8.62

Many factors such as the growing period and growing conditions of the donor plant, fertilizing, pre-treatments and genotype affect the anther culture. However, the most important factor is genotype in anther culture (Kristiansen and Andersen, 1993; Qin and Rotino, 1993; Rodeva et al., 2004; Lantos et al., 2009; Nowaczyk et al. et al., 2009; Arı et al., 2016; Durna, 2016; Atasoy, 2020). Nine different pepper genotypes produced different results in embryo formation (Shtereva et al., 1998). Similarly, success in pepper anther culture varies depending on genotypes (Keleş et al., 2015). Our results are following the previous reports, and genotype and medium effect on the fecundity in pepper anther culture.

The embryo and plantlet initiation of pepper genotypes differed depending on the medium. While genotypes resulted in success in embryo formation, there have also been genotypes with low success. Whereas the response of the same genotype to embryo formation in the different medium was high, it could not produce the same success response during the transformation into a plant and its success remained low (Table 3).

Table 3
Embryo number (EN), embryo frequency (% EF), plant number (PN), plant frequency (% PF) and conversion rate to plant (% CRP) according to genotypes and nutrient mediums

Genotypes	Mediums	EN	EF	PN	PF	CRP
KLASMAN F1	O1	1	0.88	1	0.88	100.0
	O2	1	2.08	1	2.08	100.0
	∑	2	1.23	2	1.23	100.0
FLINTA F1	O1	1	0.72	0	0.00	0.0
	O2	1	1.52	0	0.00	0.0
	∑	2	0.98	0	0.00	0.0
DORU16	O1	1	1.67	0	0.00	0.0
	O2	4	7.41	1	1.85	25.0
	∑	5	4.38	1	0.87	20,0
DOLPHIN	O1	2	0.93	1	0.46	50.0
	02	6	7.14	4	4.76	66.6
	∑	8	2.16	5	1.35	62.5
SÜ 29	O1	0	0.00	0	0.00	0.0
	O2	4	3.51	3	2.63	75.0
	∑	4	1.85	3	1.38	75.0
SÜ 30	O1	0	0.00	0	0.00	0.0
	O2	3	3.57	1	1.19	33.3
	∑	3	2.08	1	0.69	33.3
SÜ 31	O1	0	0.00	0	0.00	0.0
	02	2	4.17	1	2.08	50.0
	∑	2	1.66	1	0.83	50.0
SÜ 32	O1	1	0.52	1	0.52	100.0
	O2	2	3.03	1	1.52	50.0
	∑	3	1.16	2	0.77	66.6
SÜ 33	O1	0	0.00	0	0.00	0.0
	O2	2	2.78	1	1.39	50.0
	∑	2	1.19	1	0.59	50.0
SÜ 34	O1	0	0.00	0	0.00	0.0
	O2	0	0.00	0	0.00	0.0
	∑	0	0.00	0	0.00	0.0
SÜ 35	O1	0	0.00	0	0.00	0.0
	O2	2	2.78	1	1.39	50.0
	∑	2	1.14	1	0.57	50.0
SÜ 36	O1	0	0.00	0	0.00	0.0
	O2	4	5.56	1	1.39	25.0
	∑	4	1.90	1	0.47	25.0
	General	37	1.63	18	0.79	48.6

While the highest embryo initiation were obtained from cv. Doru 16 (1.67%), cv. Klasman F1 (0.88%) and cv. Dolphin (0.93%) in O1 medium, SU-36, SU-35, SU-34, SU-33, SU-31, SU-30 and SU-29 gave relatively lower values. Except for SU-31, genotypes that failed in embryo formation continued their anther development, but could not respond to embryo initiation, and some developing anthers only formed callus. Doru 16 (7.41%), Dolphin (7.14%) and SU-36 (5.56%) were the best in O2 medium. Although the SU-34 genotype had a rate of 10.42% in anther development, embryo transformation did not occur.

When the conversion frequencies into plants are compared according to the media, cv. Klasman F1 had the highest frequency with 0.88%, followed by cv. Dolphin (0.46%) and SÜ-32 (0.52%), the other genotypes did not produce any response in the O1 medium. The highest frequency was 4.76% in cv. Dolphin in O2 medium. Although SU-34 and cv. Flinta F1 also formed embryos, the transformation from these embryos to the plant did not occur.

Özsoy (2019) compared MS and B5 media, 209 embryos, 134 plantlets and 57 haploid plants were obtained from MS media, while B5 media produced 218 embryos,

100 plantlets, and 27 haploid plants. It is revealed that MS medium produced the most successful output. However, there are differences between our results and these findings, and this difference is thought to be due to the genotypes and modified MS and B5 media. It is possible to explain these differences between the mediums by the genotype, growing conditions and culture periods.

Previously reports indicated that genotype, media and other factors affect the success of embryo frequency and transformation of embryos into the plants in anther culture (Parra-Vega et al., 2013). Likewise, we determined that success was related to genotype, and anthers cultured in the same period showed different reactions according to genotypes (Karakullukçu and Abak, 1992; Çömlekçioğlu et al., 1999; Çömlekcioglu et al. et al., 2001; Çiner and Tıpırdamaz, 2002; Buyukalaca et al., 2004; Koleva-Gudeva et al., 2007; Taskin et al., 2011). In the present study, it was determined that the conversion rates of the embryos formed into plants were either absent or in small amounts in some genotypes. Although the embryo formation rate in pepper was between 0.5% and 12.5% in anther culture studies, some embryos could not turn into the plants and the conversion rate to plant was 0.5% (Çiner and Tipirdamaz, 2002).

The percentage of development from a total of 1362 anthers cultured in O1 medium was 6.09%, and the embryo frequency was 0.44%, and the plant transformation frequency was 0.22%. Local genotypes 151 and 171 showed positive responses on androgenic embryo formation in MS-based B-series media with a combination of 4 mg/L NAA + 0.1mg/L BAP (Al Remi, 2013). B series media showed similar results with our O1 medium, but the O2 medium gave more positive results. This difference is thought to be due to the different $AgNO_3$ or BAP doses. Moreover, 0.25% activated charcoal had a synergistic effect on embryo initiation depending on the genotype. The percentage of anther developing from 828 anthers cultured in O2 medium was 13.53, and the embryo frequency was 3.74% (Table 4).

Table 4

The number of anthers (NA), the number of developing and callus-forming anthers (NC), the percentage of developing anthers (% PDA), the number of embryos (EN), the embryo frequency (% EF), the number of plants (PN) and plant frequency (% PF)

Media	NA	NC	PDA	EN	EF	PN	PF
O1	1362	83	6.09	6	0.44	3	0.22
O2	828	112	13.53	31	3.74	15	1.81

Transformation frequency from embryos to plant was observed as 1.81%. The O2 media gave much better results than the O1 medium. Although many different plant growth regulators were used in pepper anther culture studies, it was accepted by the researchers that the highest success was obtained from the MS basic nutrient medium using 4 mg/L NAA + 0.1 mg/L BAP + 2.5 mg/L activated carbon. (Çömlekcioglu et al., 2001; Al Remi, 2013). Our findings revealed that the androgenic response of each genotype may vary according to the media. Similar to our findings, the haploidy frequency differed with the media and the genotypes, and the highest androgenic response was determined in the DDVX for the İstek F1 and Al Kırmızı F1, in the MS medium for the Balca F1, and in the B5 medium for the Hızır F1 (Özsoy, 2019).

The ploidy levels of the plants were determined by stomatal (stoma size, number of chloroplasts and number of stomata per unit area) observations (Table 5).

Table 5

Comparison of haploid and doublehaploid plants

	Haploid	Diploid
The average number of chloroplasts in guard cells	7.2	10.4
Number of stomata (mm^2)	237.4	172.9
Average stoma length and width	19.08 - 13.81 µm	30.4 - 19.21 µm

The mean stomatal dimensions (length and width) were 30.4 µm and 19.21 µm in doublehaploids, while these values were 19.08 µm and 13.81 µm in haploids. The average number of chloroplasts in guard cells was 7.2 in haploid plants and 10.4 in doublehaploid plants. While the number of stomata per unit area (mm^2) was 172.9 in diploids and 237.4 in haploids (Figure 4), doublehaploid plants formed larger leaves and plants than haploids (Figure 5).

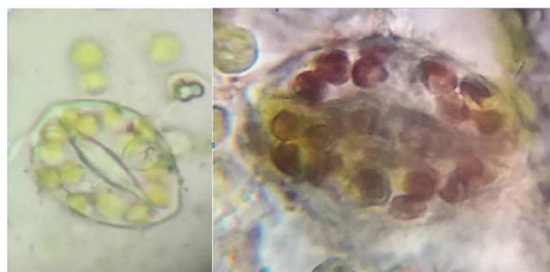

Figure 4
Stomata of haploid (left) and diploid (right) plants (400x)

Figure 5
Haploid (left) and doublehaploid (right) plants

As a result of the stomatal observations (stoma sizes, number of chloroplasts, number of stomata per unit area) of the 18 plants, 13 plants were found to be haploid (n=x=12) and 5 of them were diploid (2n=2x=24). The haploid production efficiency (HPE) was determined according to the criteria of 100 anther/haploid plants (% HPE) and 100 plant/haploid plants (% HPE). SU-29 and Klasman F1 had the highest % HPE values (Table 6).

These results reflected that the success of the anther culture technique is highly correlated with the genotype. Healthy doublehaploid shoots were obtained by applying 0.5% colchicine to the shoot tips and axillary shoots of

the 13 haploid plants propagated by micro cuttings, and fruits and pure seeds were obtained by selfing.

Table 6
Number of anthers cultured (AN), number of plants (PN), number of haploid plants (HPN), number of diploid plants (DPN), haploid production efficiency (% HPE)

Genotypes	AN	PN	HPN	DPN	% HPE
Klasman F1	162	2	2	0	1.23
Flinta F1	204	0	0	0	0.00
Doru 16	114	1	0	1	0.00
Dolphin	370	5	3	2	0.81
SÜ-29	216	3	3	0	1.38
SÜ-30	144	1	1	0	0.69
SÜ-31	120	1	1	0	0.83
SÜ-32	258	2	1	1	0.38
hSÜ-33	168	1	0	1	0.00
SÜ-34	120	0	0	0	0.00
SÜ-35	174	1	1	0	0.57
SÜ-36	210	1	1	0	0.47
∑	2260	18	13	5	0.57

4. Acknowledgment

This study is supported by Selcuk University Scientific Research Projects Coordination Unit (SU-BAP) (Project No: 19201087) Turkey. This study was prepared from the MsC Thesis of Münevver İlhan. Authors would like to thank Prof. Dr. Önder Türkmen, Assoc.Prof.Dr. Musa Seymen and internship students in Horticulture Department of Agricultural Faculty of Selcuk University.

5. Conclusion

In terms of shedding light on future studies; to obtain high haploidy frequency firstly donor plants should be grown under controlled conditions with intensive care. Keeping the number of anthers in culture as high as possible will increase the success. Refreshing the medium at certain stages of the culture, and the addition of activated charcoal are other important issues. Crossbreeding can be another solution to make unproductive genotypes productive. There is a need for new studies with the belief that making the embryo growth medium composition more effective to increase the success rate in the transformation of embryos into plants will increase the success.

6. References

Al Remi F (2013). Biberde (*Capsicum annuum* L.) anter kültüründe genotip ve besin ortamının etkisi. Çukurova Üniv. Fen Bilimleri Enstitüsü Yüksek Lisans tezi, 55 s.

Al Remi F, Taşkın H, Sönmez K, Büyükalaca S, Ellialtıoğlu Ş (2014). Effect of genotype and nutrient medium on anther culture of pepper (*Capsicum annuum* L). *Turk. J. Agric. Nat. Sci. 1*, 108-116.

Arı E, Bedir H, Yildirim S, Yildirim T (2016). Androgenic responses of 64 ornamental pepper *(Capsicum annuum L.)* genotypes to shed-microspore culture in the autumn season. *Turkish*

Journal of Biology 40 (3): 706-717.

Atasoy D (2020). Bazı Biber *(Capsicum annuum L.)* Islah Genotiplerinin Anter Kültürü Performansla rının Belirlenmesi. Yüksek Lisans Tezi, *Çukuro-va Üniversitesi*, Adana.

Barroso P, Rêgo M, Rêgo E, Soares W (2015). Embryogenesis in the anthers of different ornamental pepper *(Capsicum annuum L.)* genotypes. *Genetics and Molecular Res.* 14 (4): 13349-13363.

Buyukalaca S, Kilic N, Comlekcioglu N, Abak K.,Ekbic E (2004). Effects of silver nitrate and donor plant growing conditions on production of pepper *(Capsicum annuum L.)* haploid embryos via anther culture. *European J. of Hort. Sci.* 69:206-209.

Çiner DÖ, Tıpırdamaz R (2002). The effects of cold treatment and charcoal on the *in vitro* androgenesis of pepper *(Capsicum annuum L.)*. *Turkish J. of Bot.* 26 (3): 131-139.

Çömlekcioglu N, Buyukalaca, S, Abak K (2001). Effect of silver nitrate on haploid embryo induction by anther culture in pepper *(Capsicum annuum L.)*. *XIth EUCARPIA meeting on geneticsand breeding of capsicum and eggplant, Antalya, Turkey*, 133-136.

Çömlekçioğlu N, Büyükalaca S, Abak K (1999).Şanlıurfa ve Kahramanmaraş biber populasyonlarında anter kültürü yöntemiyle haploid bitki elde etme olanakları. *Türkiye III. UlusalBahçe Bit. Kong. Ankara*, 897-900.

Dumas de Vaulx RD (1989). Haploidy and pepperbreeding: a review. *Capsicum News.* (8-9): 13-17.

Dumas de Vaulx R, Chambonnet D, Pochard E (1981). *In vitro* anther culture in red pepper *(Capsicum annuum L.)*: improvement of the rateof plant production in different genotypes by treatments at 35 °C. *Agronomie*, 1: 859-864.

Durna P (2016). Bazı Biber Genotiplerinde *In Vitro* Androgenesis Uygulamalarının Dihaploid Hat Geliştirmeye Etkisi ve Dihaploid Hatların Morfolojik Karakterizasyonu. Doktora Tezi, *Tokat Gaziosmanpaşa Üniversitesi*, TOKAT.

Ellialtıoğlu Ş., Kaplan, F. ve Abak, K., 2001. The effect of carrot extract and activated charcoal on the androgenesis of pepper. *Proceedings of XIth EUCARPIA Meeting on Genetics and Breeding of Capsicum & Eggplant*, 9-13.

FAO (2019). http://www.fao.org/faostat/ ErişimTarihi 15.02.2021.

George L, Narayanaswamy S (1973). Haploid Capsicum through experimental androgenesis. *Protoplasma*, 78 (4): 467-470.

Irikova T, Grozeva S, Rodeva V (2011b). Anther culture in pepper *(Capsicum annuum L.) in vitro*. *Acta Phys. Plantarum*, 33 (5): 1559-1570.

Irikova T, Grozeva S, Popov P, Rodeva V, Todorovska E (2011a). In Vitro Response of Pepper Anther Culture *(Capsicum annuum L.)* Depend-ing on Genotype, Nutrient Medium and Duration of Cultivation. *Biotechnology & Biotec. Equipment*, 25 (4): 2604-2609.

Karakullukçu Ş, Abak K (1992). Bazı patlıcan çeşitlerinin anter kültürüne tepkileri. *Ankara Üniversitesi Ziraat Fakültesi Yıllığı*, 42: 7-12.

Keleş D, Pınar H, Ata A, Taşkın H, Yıldız S, Büyükalaca S (2015). Effect of pepper types on obtaining spontaneous doubled haploid plants via anther culture. *HortScience*, 50 (11): 1671-1676.

Kim M, Jang IC, Kim JA, Park EJ, Yoo, M, Lee Y (2008). Embryogenesis and plant regeneration ofhot pepper *(Capsicum annuum L.)* through isolated microspore culture. *Plant Cell Reports*, 27(3): 425-434.

Koleva-Gudeva LR, Spasenoski M, Trajkova F (2007). Somatic embryogenesis in pepper anther culture: The effect of incubation treatments and different media. *Sci. Hort.* 111 (2): 114-19.

Kristiansen K, Andersen SB (1993). Effects of donor plant temperature, photoperiod, and age on anther culture response of *Capsicum annuum L..Euphytica* 67 (1): 105-109.

Lantos C, Juhász AG, Somogyi G, Ötvös K, Vági P, Mihály R, Kristóf Z, Somogyi N, Pauk J (2009). Improvement of isolated microspore culture of pepper *(Capsicum annuum L.)* via co-culture with ovary tissues of pepper or wheat. *Plant Cell,Tiss. and Organ Cult.* 97 (3): 285-293.

Nowaczyk P, Olszewska D, Kisiała A (2009). Individual reaction of Capsicum F2 hybrid genotypes in anther cultures. *Euphytica*, 168 (2):225-233.

Özsoy B (2019), Genotip Besin Ortamı ve Stres Uygulamalarının Biberde Androgenesis Üzerine Etkileri. Yüksek Lisans Tezi, *Tokat Gaziosmanpaşa Üniversitesi*, TOKAT.

Parra-Vega V, Renau-Morata B, Sifres A, Seguí-Simarro JM (2013). Stress treatments and in vitro culture conditions influence microspore embryo genesis and growth of callus from anther walls of sweet pepper (*Capsicum annuum L.*). *Plant Cell, Tiss. and Organ Cult.* 112 (3): 353-360.

Qin X, Rotino G (1993). Anther culture of several sweet and hot pepper genotypes. *International Symp. on Cultivar Improvement of Hort. Crops.Part 1: Vegetable Crops* 402: 313-316.

Rodeva V, Cholakov T (2006). Influence of some climatic factors in the period of donor plants growing on responsiveness of pepper anthers to embryogenesis. *The international conference haploids in higher plants III, Vienna, Austria*, 12-15.

Shtereva L, Zagorska N, Dimitrov B, Kruleva M, Oanh H (1998). Induced androgenesis in tomato (*Lycopersicon esculentum* Mill). II. Factors affecting induction of androgenesis. *Plant Cell Reports*, 18 (3): 312-317.

Taskin H, Büyükalaca S, Keles D, Ekbiç E (2011). Induction of microspore-derived embryos by anther culture in selected pepper genotypes. *Afr. J. of Biotec.*, 10 (75): 17116-17121.

TÜİK (2020). http://www.tuik.gov.tr Erişim Tarihi: 15.02.2019.

Wang YY, Sun CS, Wang CC (1973). The induction of the pollen plantlets of triticale and *Capsicum annuum* from anther culture. *Scientia Sinica* 16 (1): 147-151.