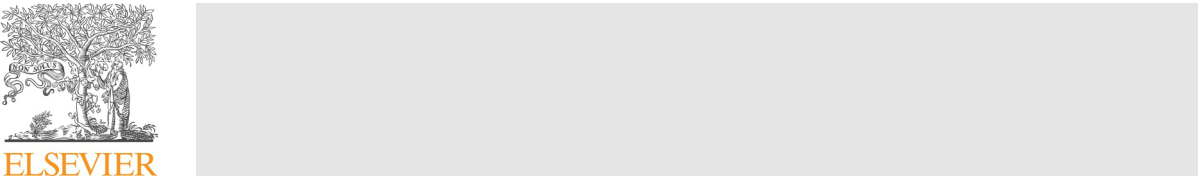
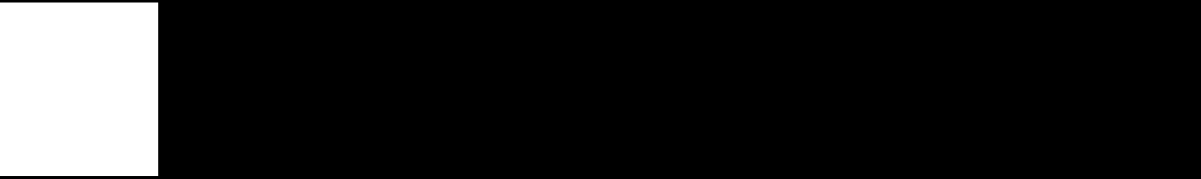
[Scientia Horticulturae 272 (2020) 109472](https://doi.org/10.1016/j.scienta.2020.109472)



Contents lists available at [ScienceDirect](http://www.sciencedirect.com/science/journal/03044238)



Scientia Horticulturae

journal homepage: [www.elsevier.com/locate/scihorti](https://www.elsevier.com/locate/scihorti)

Triple synergistic effect of maltose, silver nitrate and activated charcoal on [T](http://crossmark.crossref.org/dialog/?doi=10.1016/j.scienta.2020.109472&domain=pdf) high embryo yield of eggplant (*Solanum melongena* L.) anther cultures 



Gulsun Elif Vural[a](#page1), Esin Ari[b](#page1),[\*](#page1)

1. *Antalya Tarim Co. R & D Center, Antalya, Turkey*
2. *Department of Agricultural Biotechnology, Faculty of Agriculture, Akdeniz University, Antalya, Turkey*

ARTICLE INFO

*Keywords:*

Androgenesis

Aubergine

Biotechnology

Haploid

Microspore embryogenesis

Plant breeding

ABSTRACT

More efficient haploidy protocols have the power to speed up breeding studies. We aimed to increase micro-spore-derived embryo and *in vitro* plantlet yields in eggplant anther cultures. To achieve this, a comprehensive optimization study was carried out on the commonly used two-step DDV (Dumas de Vaulx et al., 1982) eggplant anther culture system. For optimizations of both induction and differentiation culture media, the effects of carbohydrate sources (maltose and sucrose) were investigated alone or in combination with two kinds of ad-ditives consisting of silver nitrate (AgNO3) and activated charcoal (AC). They were done in the presence of macro-micro salts, vitamins and PGRs of original DDV protocol. During the optimizations, we also compared the effects of genotype, the growth season of donor plants, anther culture type, and culture media, on embryo and*in* *vitro* plantlet yields. Regarding culture media, single effect of maltose use was considerable only at the highestconcentration. The effects of maltose + AgNO3 combinations were not noteworthy while the effect of maltose + AC combination was negative. However, triple use of maltose (90 g/L) + AgNO3 (10 mg/L) + AC (1 g/L) had a remarkably positive synergistic effect depending on the genotype, season, and concentrations of optimization chemicals. We observed a triple synergistic effect of this aSC24-encoded modified induction medium on longer viability of anthers, embryo quality, direct embryogenesis, faster regeneration and increased embryo and *in vitro* plantlet yields. Through this extremely efficient modified induction medium, we achieved 3.9 times more embryo yields than the original DDV medium in two genotypes. The highest embryo and *in vitro* plantlet yields (320 embryos and 200 plantlets/100 anthers) were obtained from ‘A117’ eggplant anthers cul-tured in solid aSC24 + DDV-R combined cultures in the autumn season. In addition, as far as we know, embryos (up to 42 embryos/100 anthers) were produced for the first time in a liquid anther culture in eggplant.

**1. Introduction**

Eggplant (*Solanum melongena* L.) (2n = 24) from Solanaceae is a vegetable with a high economic value. It was the third most produced vegetable in the world in 2018 after potato and tomato with a pro-duction of 51.28 million tonnes on an area of 1.87 million ha (FAOSTAT, 2019). Eggplant is widely cultivated in Asia and the Med-iterranean basin. Its production is mainly covered by China and India with 62 % and 24 %, respectively, which is followed by Egypt and Turkey with approximately 1 % for each.

Eggplant is consumed fresh, dried, and frozen, as well as being used in making jam, pickle, sauce and salads. It has also recently attracted attention with its inclusion in dietary lists due to its fibrous structure and low caloric value. In addition, it has long been used for alternative



medicine and drug making for different treatments such as for choles-terol, diabetes, asthma, bronchitis, digestive difficulty and tumor treatment because of its different vitamin, antioxidant, alkaloid and phenolic substance contents (Rotino, 1996; Bravo, 1998; Kuo et al., [2000](#page14); Daunay et al., 1999). Eggplant is accepted as being among the top ten vegetables regarding oxygen radical absorbance capacity because of its high phenolic content (Cao et al., 1996). According to the report of Kaushik et al. (2015), among 21 different vegetables, eggplant has the most total phenolic acid content with 32 mg/100 g fresh weight, most of which is chlorogenic acid. Thus, as indicated by Saini and Kaushik [(2019)](#page14), it may be indispensable to focus on the production of specific secondary metabolites of eggplant in the future. For all these reasons, the need and demand for eggplant has gradually increased and conse-quently the number of breeding and genetic studies has increased.

*Abbreviations:* AC, activated charcoal; AgNO3, silver nitrate; DH, doubled haploid; DDV protocol, the protocol of Dumas de Vaulx and Chambonnet (1982); Mod-Cmedium, modified induction medium; Mod-R medium, modified differantiation medium; PGR, plant growth regulator

Corresponding author.

*E-mail address:* [esinari@akdeniz.edu.tr](mailto:esinari@akdeniz.edu.tr)(E.Ari).

<https://doi.org/10.1016/j.scienta.2020.109472>

Received 8 February 2020; Received in revised form 1 May 2020; Accepted 3 May 2020

Available online 07 June 2020

0304-4238/ © 2020 Elsevier B.V. All rights reserved.

*G.E. Vural and E. Ari* *Scientia Horticulturae 272 (2020) 109472*

Since the discovery of heterosis in eggplant in 1931 ([Kakizaki,](#page13) [1931](#page13)), the main breeding method in eggplant has been F1 hybrid seed breeding. In F1 hybrid breeding, firstly homozygous pure parent lines should be obtained. Their production is possible either *via* classical selfing or doubled haploid (DH) technology. In addition to many other advantages, DH technology provides speed, time, and economic savings in plant breeding by decreasing the classic 6–8 selfing generations to only one generation.

The current DH technique used in eggplant is androgenesis. The induction of androgenesis techniques includes anther culture, isolated microspore culture, and shed-microspore cultures in general. Anther culture is the most widely used technique in eggplant for DH production because of its practical applicability. The first anther culture report in eggplant was notified by Raina and Iyer (1973). Following this study, the first haploid plants were declared by the ChineseResearch-Group-of-Haploid-Breeding (1978) and by Isouard et al. (1979). Then, [Dumas](#page13) de Vaulx and Chambonnet (1982) and Chambonnet (1985) made great improvements in the haploid plant yield of eggplant (Rotino, 2016). They formed the basis of a reproducible and reliable protocol. Still, the protocols currently used for eggplant anther cultures are based on dif-ferent versions of the Dumas de Vaulx and Chambonnet (1982) protocol developed from a similar protocol used for pepper anther culture (Segui-Simarro et al., 2011; Rotino, 2016). As another androgenesis technique, isolated microspore culture has important advantages such as high embryo yield efficiency and the elimination of the risk of forming somatic regenerants (Segui-Simarro, 2016). Until recently, the use of isolated microspore culture was quite limited in eggplant. The first striking improvement was recorded byCorral-Martinez and Segui-Simarro (2012). After the delicacy studies, the highest microspore embryogenesis rate (7.6 plants per 100 cultured calli) was reported by Rivas-Sendra et al. (2015). These results were obtained by indirect microspore embryogenesis in the presence of the callus phase followed by organogenesis. However, an isolated microspore culture protocol for direct embryogenesis in eggplant is still missing. As an alternative sub-method between anther and isolated microspore culture, the shed-mi-crospore technique has recently attracted attention in androgenesis studies. The basis of this technique is based on a double-layered anther culture system used by Johansson et al. (1982) and Dolcet-Sanjuan et al. (1997). Supena et al. (2006) modifed this technique for In-donesian hot peppers. Also, Ari et al. (2016a; [2016b](#page13)) verified that it can be used successfully in ornamental peppers. Apparently, shed-micro-spore culture seems to be an effective androgenesis technique for dif-ferent pepper types in general. However, the information is lacking for shed-microspore culture in eggplant. Therefore, it might be adapted for eggplant, as the relative of pepper, since shed-microspore culture has a higher embryo yield potential than anther culture and is more practical than isolated microspore culture.

There are several studies on the factors affecting the success of eggplant anther culture studies such as genotype (Tuberosa et al., 1987; Karakullukcu, 1991; Rotino et al., 2005; Alpsoy and Seniz, 2007; [Salas](#page14) et al., 2011; Basay and Ellialtıoglu, 2013; Rivas-Sendra et al., 2017), donor plant growth conditions (Karakullukcu, 1991; Alpsoy and Seniz, [2007](#page13)), growth season (Vural et al., 2019), determination of suitable microspore stage and bud morphology (Ryana and Iyer, 1973; Karakullukcu and Abak, 1993a; Salas et al., 2012), culture media (Research-Group-of-Haploid-Breeding, 1978; Dumas de Vaulx and Chambonnet, 1982; Chambonnet, 1985; Karakullukcu and Abak, [1993b](#page14); Rotino, 1996), shock treatment and culture conditions ([Dumas](#page13) [de Vaulx and Chambonnet, 1982](#page13); [Ellialtıoglu and Tipirdamaz, 1999](#page13)).

In this study, we aimed to increase haploid embryo and *in vitro* plantlet yields of eggplant anther cultures by optimizing both induction and differentiation culture media, which are commonly used in two-step eggplant anther culture, and also by comparing the effects of the growth season, genotype, and culture type.

**2. Materials and methods**

*2.1. Plant materials*

The present study was performed in both spring and autumn in Antalya, Turkey, to compare the seasonal effect on the androgenesis response of eggplant anthers. The donor plants; ‘A117’ (Vilmorin Seed Company), ‘Anamur’ (Rijk Zwaan Seed Co.) and ‘Darko’ (Semillas Fito Seed Co.) F1 hybrid cultivars were used in spring while ‘A117’ and ‘Anamur’ in autumn. The seeds were provided by Antalya Tarim Co. R& D Center, Antalya. Twenty seedlings for each genotype were planted in the soil in a fan-pad ventilated greenhouse on 4 April in spring and on 5 September in autumn. The recorded min-average-max temperatures of the greenhouse were 7-28−32 °C in spring and 8−22−30 °C in au-tumn. The average sun light duration and light intensity were 7.8 h and 13.000 lx in spring and 6.7 h and 9.500 lx in autumn. The plants were grown by regular irrigation - fertilization, but without exposure to any chemical or stress factor.

Microspore developmental stages of the suitable flower buds were determined by the 4’,6-diamidino-2-phenylindole (DAPI) staining method according to Custers (2003). After the first flowers were re-moved from the donor plants, appropriate buds were collected from 7 a.m. to 8:30 a.m. between 2 May and 30 July in spring and between 1 October and 7 December in autumn. We used the buds with greenish yellow anthers containing mostly late unicellular and early bicellular microspores. For sterilization, the flower buds were subjected to 70 % ethanol for 1 min and rinsed once with sterile ddH2O, and then with 15

* commercial bleach containing a few drops of Tween-20 for 15 min, and finally rinsed three times.

*2.2. Anther cultures and plant regeneration*

In addition to genotypic and seasonal effects, the effects of different anther culture types and also culture media for both the induction and differentiation stages on the yields of microspore-derived embryos and *in vitro* plantlets were compared. Different anther culture types; solid(actually semi-solid), liquid and double-layered anther culture (shed-microspore culture) were compared in spring, but only solid and liquid anther cultures in autumn. In general, a two-stage culture medium system (known as DDV protocol) consisting of induction and differ-entiation media improved by Dumas de Vaulx and Chambonnet (1982) is commonly used in eggplant anther cultures. The induction medium known as DDV-C medium and the differentiation medium known as DDV-R medium in the original DDV protocol were also used in this study as the controls of induction and differentiation media. The ori-ginal DDV-C medium consists of 120 g/L sucrose, 5 mg/L 2,4-D, 5 mg/L kinetin and 8 g/L agar in addition to protocol-specific macro and micro salts, and vitamins. As for the original DDV-R medium, it contains 30 g/ L sucrose, 0.1 mg/L kinetin and 8 g/L agar as well as the protocol-specific macro and micro salts and vitamins. The details of the cultures used in the spring and autumn seasons are given below:

*2.2.1. Spring anther cultures*

The anthers of ‘A117’, ‘Anamur’ and ‘Darko’ F1 were cultured in solid, liquid and double-layered anther culture types in spring. For each culture type, both the original induction and differentiation media were modified by the use of different concentrations of silver nitrate (AgNO₃) (0, 5, 10 mg/L), and activated charcoal (AC) (0, 1 g/L) alone or to-gether. Nevertheless, the amounts of macro and micro salts, vitamins and plant growth regulators (PGRs) in both media were kept constant in accordance with the original DDV media. So, an optimization set con-sisting of six modified media was initially established for each of the induction and differentiation stages. Furthermore, a second set was composed by only replacing the carbohydrate source of the first set, namely sucrose, with maltose again for both induction and differ-entiation. The maltose concentration was 60 g/L and 20 g/L in the new

2

|  |
| --- |
| 3 |

**Table 1**

Components of modified induction (Mod-C) and differentiation (Mod-R) media used for optimization of solid, liquid and double-layered (shed-microspore) eggplant anther cultures in spring and autumn.



|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Mod-C medium contents |  |  |  |  |  | Mod-R medium contents |  |  |  |  |  |  |
| Codes of | Codes of | Chemicals used for optimization | | |  | Codes of Mod-R media | Codes | Chemicals used for optimization | | |  |  |
| Mod-C | Mod-C | Carbohydrate source | | Additives |  | used in spring \*\*\* | of corresponding | Carbohydrate source | | Additives |  |  |
| media used in | media |  |  | Mod-R media |  |  |
| spring\* | used in autumn\*\* | Sucrose | Maltose | Silver nitrate | Activated charcoal |  | used in autumn | Sucrose | Maltose | Silver nitrate | Activated charcoal |  |
|  |  |  | \*\*\*\* |  |
|  |  | (*g/L*) | (*g/L*) | (*mg/L*) | (*g/L*) |  |  | (*g/L*) | (*g/L*) | (*mg/L*) | (*g/L*) |  |
| sSC1, sLC1, sSMC1x | aSC1, aLC1x | 120 | – | – | – | sR1y | aSR1, aLR1y | 30 | – | – | – |  |
| sSC2, sLC2, sSMC2 | aSC2, aLC2 | 120 | – | 5 | – | sR2 | aSR2, aLR2 | 30 | – | 5 | – |  |
| sSC3, sLC3, sSMC3 | aSC3, aLC3 | 120 | – | 10 | – | sR3 | aSR3, aLR3 | 30 | – | 10 | – |  |
| sSC4, sLC4, sSMC4 | aSC4 | 120 | – | – | 1 | sR4 | aSR4 | 30 | – | – | 1 |  |
| sSC5, sLC5, sSMC5 | aSC5 | 120 | – | 5 | 1 | sR5 | aSR5 | 30 | – | 5 | 1 |  |
| sSC6, sLC6, sSMC6 | aSC6 | 120 | – | 10 | 1 | sR6 | aSR6 | 30 | – | 10 | 1 |  |
|  | aSC7, aLC4 | – | 30 | – | – |  | aSR7, aLR4 | – | 20 | – | – |  |
|  | aSC8, aLC5 | – | 30 | 5 | – |  | aSR8, aLR5 | – | 20 | 5 | – |  |
|  | aSC9, aLC6 | – | 30 | 10 | – |  | aSR9, aLR6 | – | 20 | 10 | – |  |
|  | aSC10 | – | 30 | – | 1 |  | aSR10 | – | 20 | – | 1 |  |
|  | aSC11 | – | 30 | 5 | 1 |  | aSR11 | – | 20 | 5 | 1 |  |
| sSC7, sLC7, sSMC7 | aSC12 | – | 30 | 10 | 1 | sR7 | aSR12 | – | 20 | 10 | 1 |  |
| aSC13, aLC7 | – | 60 | – | – | aSR13, aLR7 | – | 20 | – | – |  |
| sSC8 sLC8, sSMC8 | aSC14, aLC8 | – | 60 | 5 | – | sR8 | aSR14, aLR8 | – | 20 | 5 | – |  |
| sSC9, sLC9, sSMC9 | aSC15, aLC9 | – | 60 | 10 | – | sR9 | aSR15, aLR9 | – | 20 | 10 | – |  |
| sSC10, sLC10, sSMC10 | aSC16 | – | 60 | – | 1 | sR10 | aSR16 | – | 20 | – | 1 |  |
| sSC11, sLC11, sSMC11 | aSC17 | – | 60 | 5 | 1 | sR11 | aSR17 | – | 20 | 5 | 1 |  |
| sSC12, sLC12, sSMC12 | aSC18 | – | 60 | 10 | 1 | sR12 | aSR18 | – | 20 | 10 | 1 |  |
|  | aSC19, aLC10 | – | 90 | – | – |  | aSR19, aLR10 | – | 20 | – | – |  |
|  | aSC20, aLC11 | – | 90 | 5 | – |  | aSR20, aLR11 | – | 20 | 5 | – |  |
|  | aSC21, aLC12 | – | 90 | 10 | – |  | aSR21, aLR12 | – | 20 | 10 | – |  |
|  | aSC22 | – | 90 | – | 1 |  | aSR22 | – | 20 | – | 1 |  |
|  | aSC23 | – | 90 | 5 | 1 |  | aSR23 | – | 20 | 5 | 1 |  |
|  | aSC24 | – | 90 | 10 | 1 |  | aSR24 | – | 20 | 10 | 1 |  |



* For instance; sSC1: Spring-Solid Mod-C1 [(modified](#page3) induction) medium, sLC1: Spring-Liquid Mod-C1 medium, sSMC1: Spring-Shed-Microspore (Double-Layer) Mod-C1 medium.
* aSC1: Autumn-Solid Mod-C1 medium, aLC1: Autumn-Liquid Mod-C1 medium.
* sR1: Spring- Mod-R1 (modified differentiation) medium (the same differentiation medium was used for each of solid, liquid and double-layer medium).
* aSR1: Autumn-Solid Mod-R1 medium, aLR1: Autumn-Liquid Mod-R1 medium.



1. Original induction (DDV-C) medium (Dumas de Vaulx and Chambonnet, 1982) was used as the control of all Mod-C media in both spring and autumn season.
2. Original differentiation (DDV-R) medium (Dumas de Vaulx and Chambonnet, 1982) was used as the control of all Mod-R media used in both spring and autumn.

|  |
| --- |
| *G.E. Vural and E. Ari* |

|  |
| --- |
| *Scientia Horticulturae 272 (2020) 109472* |

*G.E. Vural and E. Ari*

induction media and differentiation media set, respectively. The mod-ified induction and differentiation media which we attempted to opti-mize are hereinafter referred to as Mod-C and Mod-R, respectively. Thus, a total of 12 Mod-C and 12 Mod-R medium treatments were constituted for solid, liquid and double-layered anther culture types in spring treatments ([Table 1](#page3)).

In each culture type, the anthers were first cultured in each Mod-C medium for a total of 12 days, then transferred to a corresponding Mod-R medium for continuous culture in accordance with the original DDV protocol. The contents of the solid, liquid, and both layers of the double-layered anther culture media were the same except for the so-lidifiying agent. Plant agar at a concentration of 8 g/L was added to solidify the solid anther cultures and the bottom layer of shed-micro-spore cultures. In liquid cultures, differentiation media (Mod-R or DDV-

1. were also prepared in liquid form just as in the Mod-C media. Additionally, all the liquid cultures were stationary cultures, not agi-tated on a shaker. The spring experiments were performed in a 3 × 3 × 12 (genotype x culture type x Mod-C medium) factorial design with 10 replications. One hundred anthers were cultured in 10 petri dishes (60 mm in diameter) for each treatment meaning a total of 10.800 anthers were used in the spring season, in which 1.200 anthers were cultured for each of three genotypes in each of three anther culture types.

*2.2.2. Autumn anther cultures*

Autumn anther cultures were designed according to the results of spring season. Based on the spring results, double-layered anther cul-ture treatments were excluded in autumn since no response, including anther viability, was obtained. Likewise, the use of AC in liquid cultures was excluded since it created a negative effect on both androgenic re-sponse and anther viability. Additionally, ‘Darko’ F1 was removed from autumn experiments due to seedling supply problem. For these reasons, ‘A117’ and ‘Anamur’ F1 cultivars as the genotypes and solid and liquid cultures as the anther culture types were tested in autumn.

Regarding media content, since the effectiveness of maltose used in spring was found to be remarkable, the effects of a lower (30 g/L) and a higher (90 g/L) maltose concentration were also investigated in the optimization of induction media in the autumn season. This was in addition to the same Mod-C media used in spring. Thus, the third and fourth Mod-C and consequently corresponding Mod-R optimization sets were added to the first two medium sets in autumn experiments. In all Mod-R media containing maltose in autumn, the concentration of maltose was 20 g/L as in the spring experiments ([Table 1](#page3)).

For each genotype, 200 anthers were first cultured in 20 petri dishes in each of the 24 Mod-C media in solid cultures with 12 Mod-C in liquid cultures. Before transferring the anthers from the induction medium to the differentiation medium, 200 anthers were divided into two groups. Half of them were transferred to corresponding Mod-R medium and the other half to the original DDV-R medium. Thus, the effects of two dif-ferentiation media were also compared in autumn, unlike the spring experiments in which only the effect of Mod-R type differentiation medium was investigated (Table 1). The pH of all media was adjusted to 5.8 and all media were autoclaved at 121 °C for 20 min. No media re-newal was performed in the cultures in both seasons.

The autumn experiments were performed in 2 × 24 × 2 and 2 × 12 × 2 factorial (genotype x induction medium x differentiation medium) design with 10 replications in solid and liquid cultures, re-spectively. Therefore, a total of 14.400 anthers were used in autumn of which 2.400 and 1.200 anthers were cultured in each of solid and liquid anther culture type, respectively, for each genotype.

*2.2.3. Pre-treatment and culture conditions of anther cultures*

We used almost the same pre-treatment and culture conditions as in the original DDV protocol. The anthers were first cultured in Mod-C media at 35 °C for 8 days in the dark to initiate embryogenesis, then incubated at 25 °C for an additional 4 days under 16-h light / 8-h dark

*Scientia Horticulturae 272 (2020) 109472*

(12 h/12 h in DDV protocol) photoperiod (40 μmol/m2/s) regime. On the 13th day, they were transferred to Mod-R media to differentiate induced microspores and to ensure further growth of embryos. After that, they were kept at 25 °C under photoperiod (60 μmol/m2/s) until cotyledonary or spontaneously germinating embryos appeared.

*2.2.4. Plantlet conversion and acclimatization*

For *in vitro* plantlet conversion, we used the Murashige and Skoog [(1962)](#page14) (MS) medium unlike the original DDV protocol where a multi-plication medium called V3 is used. In order to obtain healty *in vitro* plantlets, cotyledonary embryos, and also the embryos beginning to germinate inside the differentiation media, were transferred to an MS medium supplemented with 30 g/L sucrose and 8 g/L agar, without any PGR. Upon the formation of enough strong shoots and roots, *in vitro* plantlets were transferred to pots containing a mixture of peat moss, perlite and vermiculite (3:1:1 v/v/v) for acclimatization. Later, they were subjected to gradually decreasing humidification and a gradually increasing lighting regime, and then moved to a greenhouse. After they were selfed and observed in greenhouse conditions, the seeds of se-lected lines were taken and included in the breeding program.

*2.3. Ploidy analysis*

The ploidy levels of a total of 1492 *ex vitro* regenerated eggplant plants grown in the greenhouse were determined mostly by morpho-logical observations, also in part by counting the number of chlor-oplasts in the stomata guard cells and using a flow cytometer.

Initially, due to a problem with the flow cytometer, ploidy analyses of all plants were first performed using morphological observations based on the growth and rooting performance, shape of the leaves and leaf veins, and pollen formation status of the plants. For doubtful si-tuations, the method of chloroplast counting in stomata guard cells was applied to 120 plants. For this aim, the lower epidermis layer of a small piece of mature leaf was peeled by tearing with hand and placed on a microscope slide for each sample. A one drop of AgNO3 stock solution (5 mg/mL) was added with a Pasteur pipette and a coverslip was cov-ered, and immediately after, the number of chloroplasts per guard cell pair was counted under a light microscope.

After the problem in the flow cytometer was resolved towards the end of the study, the 100 ‘A117’ *ex vitro* eggplant regenerants obtained from autumn solid cultures were subjected to cytometric analysis with a small-scale sampling in order to both provide more reliable ploidy analysis results and confirm the morphological observations. For this purpose, approximately 0.5 cm2 of young leaf pieces were cut from the plants. They were processed by using a CyStain UV Precise P Kit for extraction and staining, and then analyzed in a CyFlow Ploidy Analyzer (Partec GmbH, Germany).

*2.4. Data and statistical analysis*

The factorial experiments based on a completely randomised design with ten replications were performed. Details of anther and petri numbers used in each treatment were given above. For the expression of embryo and *in vitro* plantlet yields of 3-month cultures, we used average numbers of embryo and plantlets per 100 anthers. Callus for-mation was not mentioned in this study due to another publication in preparation. Data were presented as mean ± standard error. For sta-tistics, data were subjected to analysis of variance followed by the Tukey HSD test at an alpha 0.05 level using JMP 8.0 program (SAS Institute Inc., USA).

**3. Results**

*3.1. Spring anther cultures*

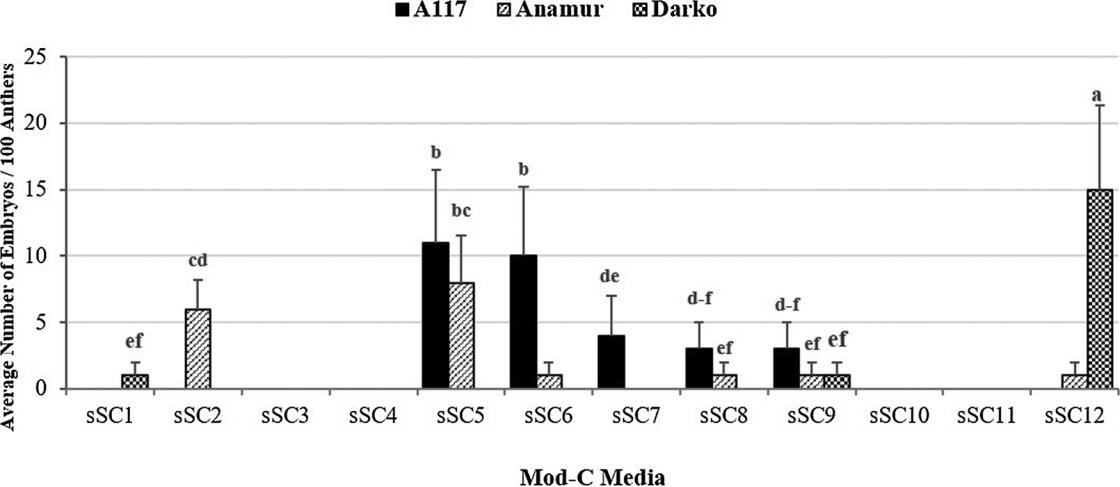
The effects of the factors consisting of genotype (‘A117’, ‘Anamur’

4

*G.E. Vural and E. Ari*

and ‘Darko’ F1 cultivars), induction medium (12 Mod-C) and anther culture type (solid, liquid and double-layered) on embryo and *in vitro* plantlet yields of eggplant anther cultures were investigated in the spring season. The first embryo formation was observed in sucrose media on the 35th day of the culture and in maltose media on the 45th day in solid cultures. According to the variance analyses of spring an-ther cultures (data not shown), the effect of genotype, as independent from the effects of other factors consisting of induction medium and anther culture type, on embryo (*p* = 0.237) and *in vitro* plantlet (*p* = 0.611) production, was not significant. However, ‘A117’ had a higher average embryo and plantlet yield than ‘Darko’ and ‘Anamur’, respec-tively. The effect of culture type was significant (*p*< 0.001) and the solid anther culture type was the single successful culture type to form embryo and consequently *in vitro* plantlet. The effect of the Mod-C medium was also significant (*p* < 0.001). The most successful three inductive Mod-C media in solid cultures were sSC5, sSC12 and sSC6, respectively. According to Table 1, all of these media contained 0.1 % (1 g/L) AC. The carbohydrate source was sucrose in sSC5 and sSC6 media, and maltose in the sSC12 medium. One other common in-gredient of these media was AgNO3. The sSC5 medium had 5 mg/L AgNO3, while sSC6 and sSC12 media contained 10 mg/L AgNO3. The interactions of the genotype x Mod-C medium and also the culture type x Mod-C medium created significant difference (*p*< 0.001) on embryo and *in vitro* plantlet yield while the genotype x culture type interaction was nonsignificant. Additionally, the interaction of the genotype x culture type x Mod-C medium was found significant (*p* < 0.001). The effect of Mod-C media on average embryo yields of solid anther cultures of ‘A117’, ‘Anamur’ and ‘Darko’ eggplant cultivars in spring is shown in Fig. 1. Regarding Fig. 1 and further graphics, it is required to underline that the yields of embryo, and therefore *in vitro* plantlets were obtained as the result of combined use of first a modified induction and then a corresponding differentiation medium. However, Mod-C media as the inductive factor for microspore embryogenesis were highlighted to provide clearer and more understandable graphics.

According to Fig. 1, the highest average embryo yield (15 embryos / 100 anthers) was obtained from the anthers of ‘Darko’ in sSC12 medium consisiting of 60 g/L maltose, 10 mg/L AgNO3 and 1 g/L AC in addition to the macro-micro salts, vitamins, and PGRs of the original DDV medium. The sSC5 and sSC6 media followed the sSC12 medium in terms of embryo production. In spring, the media containing AgNO₃ and also the combination of AgNO₃ and AC further stimulated the embryo formation and *in vitro* plantlet development. As for the effi-ciency of control medium (sSC1), which is the original DDV-C medium,



*Scientia Horticulturae 272 (2020) 109472*

it could not supply any androgenic response in ‘A117’ and ‘Anamur’. In ‘Darko’, the control medium could produce only 1 embryo/100 anthers, which means a 15 times lower yield than the aSC12 medium.

Embryo formation occured only in solid anther cultures. In liquid cultures, viability and growth of the anthers were good according to microscopic observations, but no embryo formed. The anthers in liquid cultures kept their viability and green colors until the end of the culture period, especially in the media containing the combination of maltose and AgNO3. Therefore, it was decided to also investigate the efficiencies of a lower (30 g/L) and a higher (90 g/L) dose of the previous tested maltose concentration (60 g/L) in autumn. As for the shed-microspore cultures, the colors of all cultured anthers turned brown or black and lost their viabilities during the first 8-days of incubation at 35 °C. Nevertheless, they were still transferred to corresponding Mod-R media on the 13th day of the culture like the others in solid or liquid cultures. However, further growth did not ocur in shed-microspore cultures possibly due to the excess or improper use of AC in the liquid layer of the cultures. For this reason, the shed-microspore culture type was re-moved and the use of AC was excluded from liquid anther cultures in autumn.

A total of 64 embryos, 45 *in vitro* plantlets and 45 acclimatized plants, were obtained from 3.600 anthers in solid anther cultures from the spring season. The transformation rate of embryos into *in vitro* plantlets was 70.3 % while the acclimatization success rate was 100 %. The total embryo, *in vitro* and acclimatized plant yields obtained from the anthers of ‘A117’, cultured in solid anther cultures in spring are 31, 18 and 18, respectively. The same alignment for ‘Anamur’ is 16, 11 and 11 while those of ‘Darko’ is 17,16, 16.

*3.2. Autumn anther cultures*

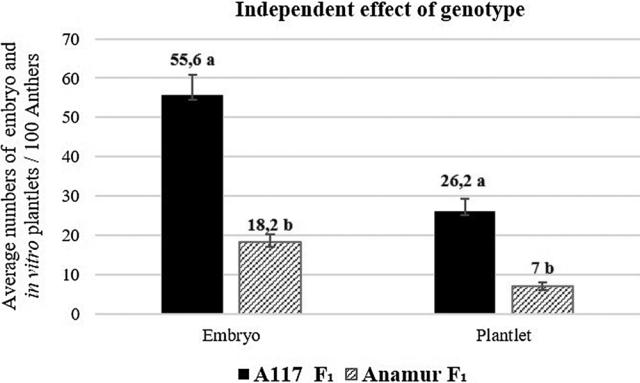
The effects of the factors consisting of genotype (‘A117’ and ‘Anamur’ F1), induction medium (24 solid and 12 liquid Mod-C) and differentiation medium (corresponding Mod-R and original DDV-R) on embryo and *in vitro* plantlet yields of eggplant anther cultures were investigated in autumn (Table 1). Interestingly, the embryo formation in autumn cultures began earlier than spring ones. The first embryo was observed in sucrose media on the 27th day of the culture and in maltose media on the 35th day in solid cultures. Solid anther cultures were much more proliferative than liquid cultures. The effects of all factors examined in solid and liquid cultures in autumn are given below se-parately since the factor of culture type, as the fourth factor, could not be evaluated statistically together with other three factors.

**Fig. 1.** Effect of modified induction (Mod-C) media on average embryo yields (embryos / 100 anthers) of ‘A117’, ‘Anamur’ and ‘Darko’ F1 eggplant cultivars in solidanther cultures of spring season (*p* < 0.001) (values are mean and ± standard error bars and means within each column followed by the same letter are not significantly different at p < 0.05).

5

*G.E. Vural and E. Ari* *Scientia Horticulturae 272 (2020) 109472*

**Fig. 2.** The independent effect of genotype factor on the average yields ofembryos and *in vitro* plantlets / 100 anthers in solid cultures of the autumn season (p < 0.001), regardless of the effect of modified induction (Mod-C) or differentiation medium (values are mean and ± standard error bars and means within each column followed by the same letter are not significantly different at p < 0.05).



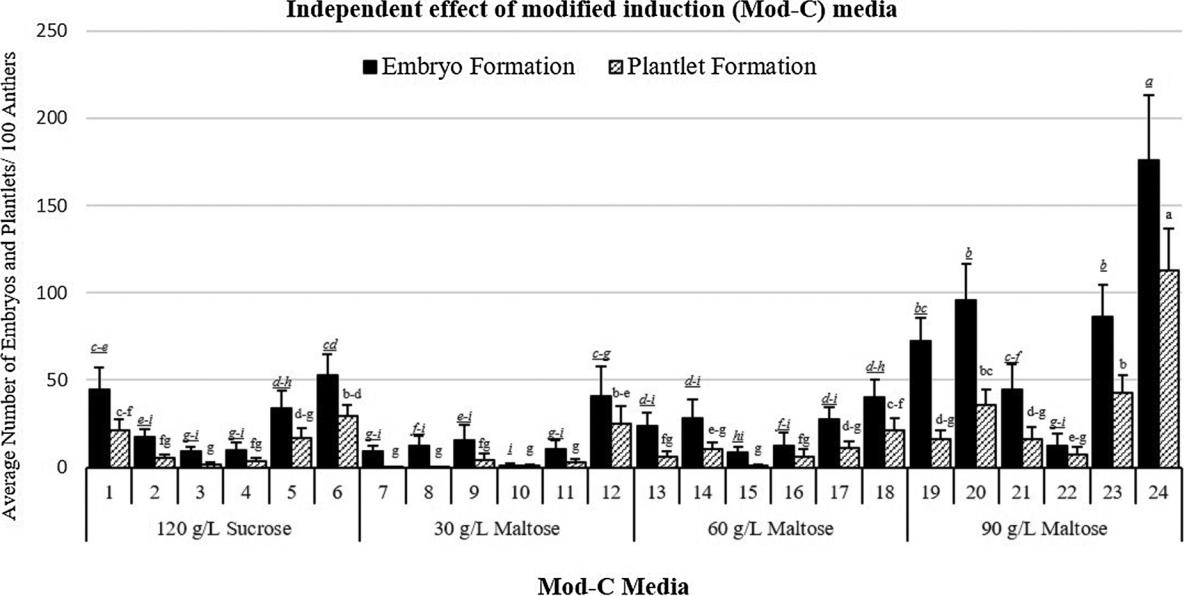
*3.2.1. Solid anther cultures*

Independent factor effects of genotype, induction and differentia-tion medium as well as their two and three-way interactions were evaluated below according to their variance analyses (data not shown).

*3.2.1.1. Independent effect of genotype*. The effect of genotype, asindependent from the effects of modified induction or differentiation medium, on embryo and *in vitro* plantlet yields in solid cultures in autumn was found significant (*p* < 0.001). ‘A117’ produced 3-fold more embryos and 3.7 times more *in vitro* plantlet yields than ‘Anamur’ (Fig. 2).

*3.2.1.2. Independent effect of modified induction (Mod-C) medium*. Regardless of the effects of genotype or differentiationmedium, the Mod-C medium had a significant (p < 0.001) effect on embryo and *in vitro* plantlet yields ([Fig. 3](#page6)).

As seen from Fig. 3, the highest embryo and *in vitro* plantlet for-mation happened in the Mod-C media group with the highest



concentration of maltose. Among Mod-C media with 90 g/L maltose, the aSC24 (the 24th Mod-C) medium was the superior in forming the highest embryo (175.8 embryos/100 anthers) and *in vitro* plantlet (112.8 plantlets/100 anthers) yields (Fig. 3). The aSC24 medium had 10 mg/L AgNO3 + 1 g/L AC in addition to macro-micro salts, vitamins and PGRs of the original DDV medium. However, the control induction medium (aSC1), formed average yields of 44.8 embryos/100 anthers and 21 *in vitro* plantlets/100 anthers. Thus, the aSC24 medium gener-ated 3.9 times more embryos and a 5.4 fold higher plantlet yield than the control medium, regardless of the effect of genotype or differ-entiation medium.

*3.2.1.3. Independent effect of differentiation medium*. After the culture ineach Mod-C medium in the first 12 days, the anthers were divided and cultured in two types of differentiation media; i) corresponding Mod-R, and ii) original DDV-R medium. The independent effect of the differentiation medium, regardless of the effect of genotype or Mod-C medium, did not cause a significant difference in the yields of embryo (*p* = 0.725) and *in vitro* plantlet (*p* = 0.791). However, Mod-R media (37.7 embryos and 17 plantlets per 100 anthers) formed slightly higher yields in general than the original differentiation (DDV-R) medium (36 embryos and 16.3 plantlets per 100 anthers) (data not shown).

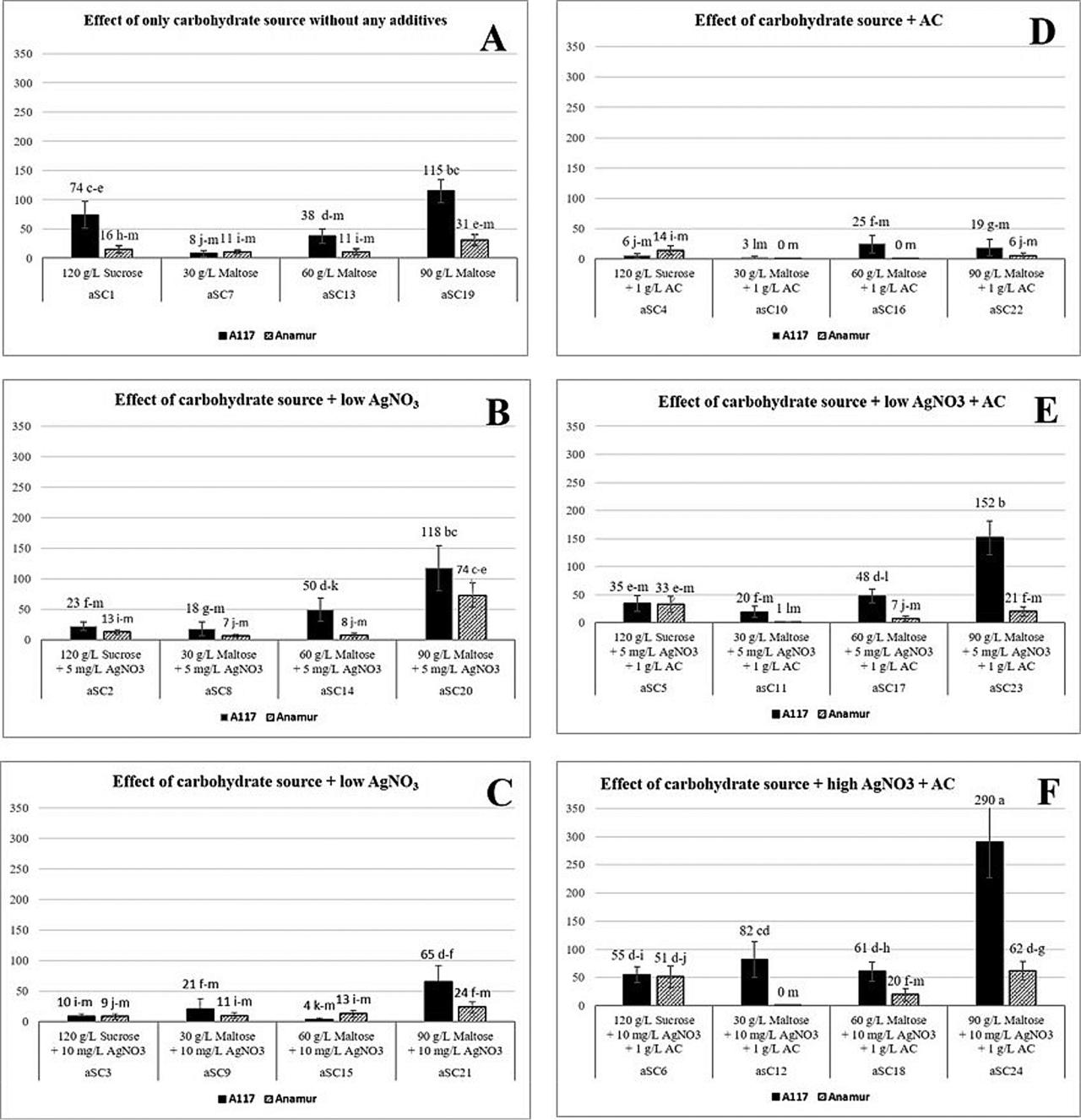
*3.2.1.4. Effects of interactions*. Regardless of the effect of differentiationmedium, the interaction of the genotype x Mod-C medium had a significant (p < 0.001) effect on embryo and *in vitro* plantlet formation. While assessing the effects of this interaction, we have reached some important conclusions about the side effects of optimization chemicals used alone or in combination. To express them in a more meaningful way, we designed Fig. 4. In fact, [Fig.4](#page7) is essentially a single graph showing embryo yields of ‘A117’ and ‘Anamur’ obtained from 24 Mod-C media aligned side by side from sSC1 to aSC24 (see supplementary material). However, in order to better illustrate the single, dual, and triple effects of the optimization chemicals, we have transformed Fig. 4 into six sub-graphs. Thus, the statistical representations in each sub-graph may appear as if they are inconsistent, but it should be noted that these are actually taken from a single graph.

According to Fig. 4, the average embryo yield of ‘A117’ is much higher than ‘Anamur’ in almost every Mod-C medium. The highest three

**Fig. 3.** The independent effect of different induction (Mod-C) media on the average yields of embryo and*in vitro*plantlets/100 anthers in solid cultures of the autumnseason (p < 0.001), regardless of the effect of genotype or differentiation medium (values are mean and ± standard error bars and means within each column followed by the same letter are not significantly different at p < 0.05).

6

*G.E. Vural and E. Ari* *Scientia Horticulturae 272 (2020) 109472*



**Fig. 4.** Average embryo yields of ‘A117’ and ‘Anamur’ anthers cultured in different induction (Mod-C) media in solid cultures of autumn season (p < 0.001), as theresult of genotype x Mod-C interaction (values are mean and ± standard error bars and means within each column of embryo and *in vitro* plantlet formation followed by the same letter are not significantly different at p < 0.05) (see supplementary material).

embryo yields in the solid autumn anther cultures eventuated in ‘A117’ anthers cultured in aSC24 (290 embryos/100 anthers), aSC23 (151.5 embryos/100 anthers) and aSC20 (118 embryos/100 anthers) media, respectively. The same alignment for ‘Anamur’ is; aSC20 (73.5 em-bryos/100 anthers), aSC24 (61.5 embryos/100 anthers) and aSC6 media (51 embryos/100 anthers), respectively. The most successful Mod-C medium, aSC24, produced 1.9 fold more embryos than aSC23, 2.4 fold more embryos than aSC20 and 3.9 fold more embryos than the control, original DDV-C medium. Thus, it can be concluded that the Mod-C media with the highest concentration (90 g/L) of maltose en-couraged embryo formation in both genotypes with the help of AgNO3 and in particular with the use of AgNO3 together with AC. The impacts of the optimization chemicals will be discussed in more detailed in the

discussion section.

Effects of the interactions of the genotype x differentiation medium and also the induction medium x differentiation medium were not significant for embryo yield. In addition, the triple interaction effect among the genotype x Mod-C medium x differentiation medium on embryo (*p* = 0.107) and *in vitro* plantlet (*p* = 0.137) yield was also not important. Nevertheless, the effects of the two types of differentiation medium (Mod-R and original DDV-R) on the average embryo and *in* *vitro* plantlet yields of ‘A117’ and ‘Anamur’ anthers cultured previouslyin 24 different Mod-C media are given in [Table 2](#page8).

According to Table 2, the highest average embryo and *in vitro* plantlet yield reached 320 embryos and 200 plantlets/100 anthers when ‘A117’ anthers were cultured first in aSC24 induction medium

7

*G.E. Vural and E. Ari* *Scientia Horticulturae 272 (2020) 109472*

**Table 2**

Effects of two types of differentiation media on average embryo and*in vitro* plantlet yields of ‘A117’ and ‘Anamur’ F1 anthers cultured previously in each of 24 Mod-C media in solid anther cultures of autumn season, as the result of triple interaction effect of genotype x induction medium x differentiation medium (p > 0.05).

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Code of Mod-C | The | Chemicals |  |  | Average embryo yield | | | | | |  |  |  |  | Average *in vitro* plantlet yield | | | | | | |  |  |  |
| medium used | number of | used for optimization | |  | (embryos / 100 anthers) | | | | | |  |  |  |  | (plantlets / 100 anthers) | | | | | |  |  |  |  |
| before differen- | anthers [\*\*](#page8) |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Carbo- hydrate | Additives (g/L) \*\*\* | | A117 F1 | | |  |  |  | Anamur F1 | |  |  | A117 F1 | | |  |  |  | Anamur F1 |  |  |  |
| tiation medium |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | Source |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  |  | AgNO3 | AC |  | Differentiation media | | | | | Differentiation media | | | |  | Differentiation media | | | | | Differentiation media | |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  | |  | |  |  | |  | |  |  | |  | |  |  |  |  |  |
|  |  |  |  |  |  | Mod-R[x](#page8) | | DDV-R | | | Mod-R | | DDV-R | | Mod-R | | | DDV-R | | | Mod-R | DDV-R |  |  |
| aSC1\* | 200 | 120 » 30 g/L | 0 | 0 | 15 | | ± 6 | 134 ± 36 | |  | 14 | ± 4 | 24 | ± 12 | 7 ± 3 | | | 63 | ± 21 |  | 4 ± 3 | 12±8 |  |  |
| aSC2 | 200 | Sucrose in | 5 | 0 | 30 | ± 12 |  | 12 | ± 6 | 10 | ± 4 |  | 1 ± 1 |  |  |
| aSC3 | 200 | Mod-C » Mod- | 10 | 0 | 8 ± 5 | | | 11 | ± 6 |  | 6 ± 5 | | 11 | ± 7 | 1 ± 1 | | | 2 ± 2 | |  | 3 ± 3 | 1 ± 1 |  |  |
| aSC4 | 200 | R medium | 0 | 1 | 4 ± 4 | | | 8 ± 5 | |  | 26 | ± 14 | 2 ± 2 | | 3 ± 3 | | | 3 ± 3 | |  | 8 ± 6 | 1 ± 1 |  |  |
| aSC5 | 200 |  | 5 | 1 | 44 | | ± 17 | 25 | ± 23 |  | 59 | ± 26 | 7 ± 6 | | 28 | | ± 14 | 9 ± 9 | |  | 30±15 | 1 ± 1 |  |  |
| aSC6 | 200 |  | 10 | 1 | 83 | | ± 23 | 27 | ± 13 |  | 72 | ± 35 | 30 | ± 16 | 57 | | ± 18 | 14 | ± 8 |  | 26±13 | 20±9 |  |  |
|  |  |  |  |  |  | | |  |  |  |  | |  |  |  | | |  | |  |  |  |  |  |
| aSC7 | 200 | 30 » 20 g/L | 0 | 0 | 4 ± 3 | | | 12 | ± 9 |  | 7 ± 3 | | 14 | ± 5 | 1 ± 0 | | | 1 ± 0 | |  | 0 ± 0 | 1 ± 0 |  |  |
| aSC8 | 200 | Maltose in | 5 | 0 | 12 | | ± 6 | 24 | ± 22 |  | 7 ± 3 | | 7 ± 3 | | 1 ± 0 | | | 1 ± 0 | |  | 0 ± 0 | 0 ± 0 |  |  |
| aSC9 | 200 | Mod-C » Mod- | 10 | 0 | 4 ± 2 | | | 37 | ± 35 |  | 11 | ± 6 | 10 | ± 5 | 1 ± 0 | | | 15 | ± 15 |  | 1 ± 1 | 0 ± 0 |  |  |
| aSC10 | 200 | R medium | 0 | 1 | 5 ± 5 | | | 1 ± 0 | |  | 0 ± 0 | | 0 ± 0 | | 4 ± 4 | | | 1 ± 0 | |  | 0 ± 0 | 0 ± 0 |  |  |
| aSC11 | 200 |  | 5 | 1 | 34 | | ± 19 | 5 ± 4 | |  | 2 ± 1 | | 0 ± 0 | | 10 | | ± 8 | 1 ± 1 | |  | 0 ± 0 | 0 ± 0 |  |  |
| aSC12 | 200 |  | 10 | 1 | 129 ± 59 | | | 34 | ± 18 |  | 0 ± 0 | | 0 ± 0 | | 80 | | ± 35 | 19 | ± 13 |  | 0 ± 0 | 0 ± 0 |  |  |
|  |  |  |  |  |  | |  |  |  |  |  |  |  | |  | | |  |  |  |  |  |  |  |
| aSC13 | 200 | 60 » 20 g/L | 0 | 0 | 20 | | ± 8 | 55 | ± 23 |  | 19 | ± 10 | 2 ± 2 | | 1 ± 0 | | | 22 | ± 13 |  | 2 ± 2 | 0 ± 0 |  |  |
| aSC14 | 200 | Maltose in | 5 | 0 | 99 | | ± 32 | 1 ± 0 | |  | 4 ± 2 | | 11 | ± 6 | 41 | | ± 12 | 1 ± 0 | |  | 0 ± 0 | 1 ± 1 |  |  |
| aSC15 | 200 | Mod-C » Mod- | 10 | 0 | 1 ± 0 | | | 7 ± 5 | |  | 23 | ± 10 | 3 ± 3 | | 1 ± 1 | | | 1 ± 0 | |  | 1 ± 1 | 2 ± 2 |  |  |
| aSC16 | 200 | R medium | 0 | 1 | 4 ± 3 | | | 46 | ± 28 |  | 0 ± 0 | | 0 ± 0 | | 2 ± 1 | | | 23 | ± 17 |  | 0 ± 0 | 0 ± 0 |  |  |
| aSC17 | 200 |  | 5 | 1 | 57 | | ± 15 | 38 | ± 20 |  | 14 | ± 9 | 1 ± 1 | | 20 | | ± 9 | 20 | ± 10 |  | 4 ± 3 | 1 ± 1 |  |  |
| aSC18 | 200 |  | 10 | 1 | 35 | | ± 12 | 86 | ± 30 |  | 39 | ± 21 | 0 ± 0 | | 16 | | ± 8 | 50 | ± 21 |  | 20±14 | 0 ± 0 |  |  |
|  |  |  |  |  |  | |  |  | |  |  |  |  |  |  | | |  |  |  |  |  |  |  |
| aSC19 | 200 | 90 » 20 g/L | 0 | 0 | 96 | | ± 30 | 133 ± 27 | |  | 12 | ± 7 | 49 | ± 17 | 6 ± 3 | | | 42 | ± 16 |  | 2 ± 1 | 16±7 |  |  |
| aSC20 | 200 | Maltose in | 5 | 0 | 123 ± 57 | | | 113 ± 49 | |  | 47 | ± 29 | 100 ± 26 | | 39 | | ± 18 | 51 | ± 28 |  | 7 ± 4 | 45±16 |  |  |
| aSC21 | 200 | Mod-C » Mod- | 10 | 0 | 86 | | ± 49 | 44 | ± 25 |  | 39 | ± 14 | 9 ± 7 | | 27 | | ± 21 | 24 | ± 18 |  | 13±5 | 2 ± 2 |  |  |
| aSC22 | 200 | R medium | 0 | 1 | 4 ± 3 | | | 34 | ± 27 |  | 0 ± 0 | | 11 | ± 10 | 2 ± 2 | | | 20 | ± 17 |  | 0 ± 0 | 7 ± 7 |  |  |
| aSC23 | 200 |  | 5 | 1 | 133 ± 39 | | | 170 ± 47 | |  | 35 | ± 15 | 6 ± 6 | | 85 | | ± 28 | 63 | ± 20 |  | 18±7 | 5 ± 5 |  |  |
| aSC24 | 200 |  | 10 | 1 | 260 ± 84 | | | 320 ± 98 | |  | 94 | ± 21 | 29 | ± 22 | 170 ± 57 | | | 200 ± 63 | |  | 67±13 | 14±9 |  |  |
|  |  |  |  |  |  | |  |  |  |  |  |  |  |  |  | |  |  |  |  |  |  |  |  |
| Mean: | 200 |  |  |  | 55 | | ± 7 | 58 | ± 8 |  | 23 | ± 3 | 14 | ± 2 | 26 | | ± 4 | 27 | ± 4 |  | 9 ± 1 | 5 ± 1 |  |  |
|  |  |  |  |  |  |  |  |  | | |  |  |  |  |  | | |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  | (*p* = 0.107) | | |  |  |  |  | (*p* = 0.137) | | |  |  |  |  |  |  |  |

* Control medium, where original induction and differentiation medium of DDV ([Dumas de Vaulx and Chambonnet,](#page13) 1982) medium were used.
* 200 anthers were cultured in each Mod-C media for each cultivar for a total of 12 days. Then, 200 anthers were divided into two; half of them were transffered to corresponding Mod-R while the other half were cultured in the original DDV-R medium.
* Additives were used in both Mod-C and Mod-R media in the same amount.

x Values are mean (M) and ± standard error (SE).

and then in the original DDV-R differentiation medium. When the an-thers of ‘A117’ were transferred from the aSC24 medium to the corre-sponding Mod-R differentiation medium, the average embryo and *in* *vitro* plantlet yields were 260 embryos and 170 plantlets/100 anthers,as the second highest yield. In ‘Anamur’, the highest average embryo yeild was 100 embryos/100 anthers when anthers were previously cultured in aSC20, then in the DDV-R medium. This value was followed by 94 embryos/100 anthers as the result of the treatment in which anthers were cultured first in aSC24 and then in the corresponding Mod-R medium. In terms of *in vitro* plantlet yields of ‘Anamur’, the highest average yield (67 plantlets/100 anthers) occurred in the culture combination of the aSC24 medium and the corresponding Mod-R medium. According to the mean values in Table 2, in general, the ori-ginal DDV-R differentiation medium (58 embryos and 27 plantlets/100 anthers) produced slightly higher embryo and plantlet yields than the Mod-R medium (55 embryos and 25 plantlets/100 anthers) in ‘A117’. In the case of ‘Anamur’, the Mod-R differentiation medium (22 embryos and 9 plantlets/100 anthers) procured higher embryo and plantlet yields than the original DDV-R medium (14 embryos and 5 plantlets/ 100 anthers).

A total of 3.544 embryos, 1.604 *in vitro* plantlets, and 1.445 accli-matized plants were obtained from 9.600 anthers in solid cultures of the autumn season. The transformation rate of embryos to *in vitro* plantlets

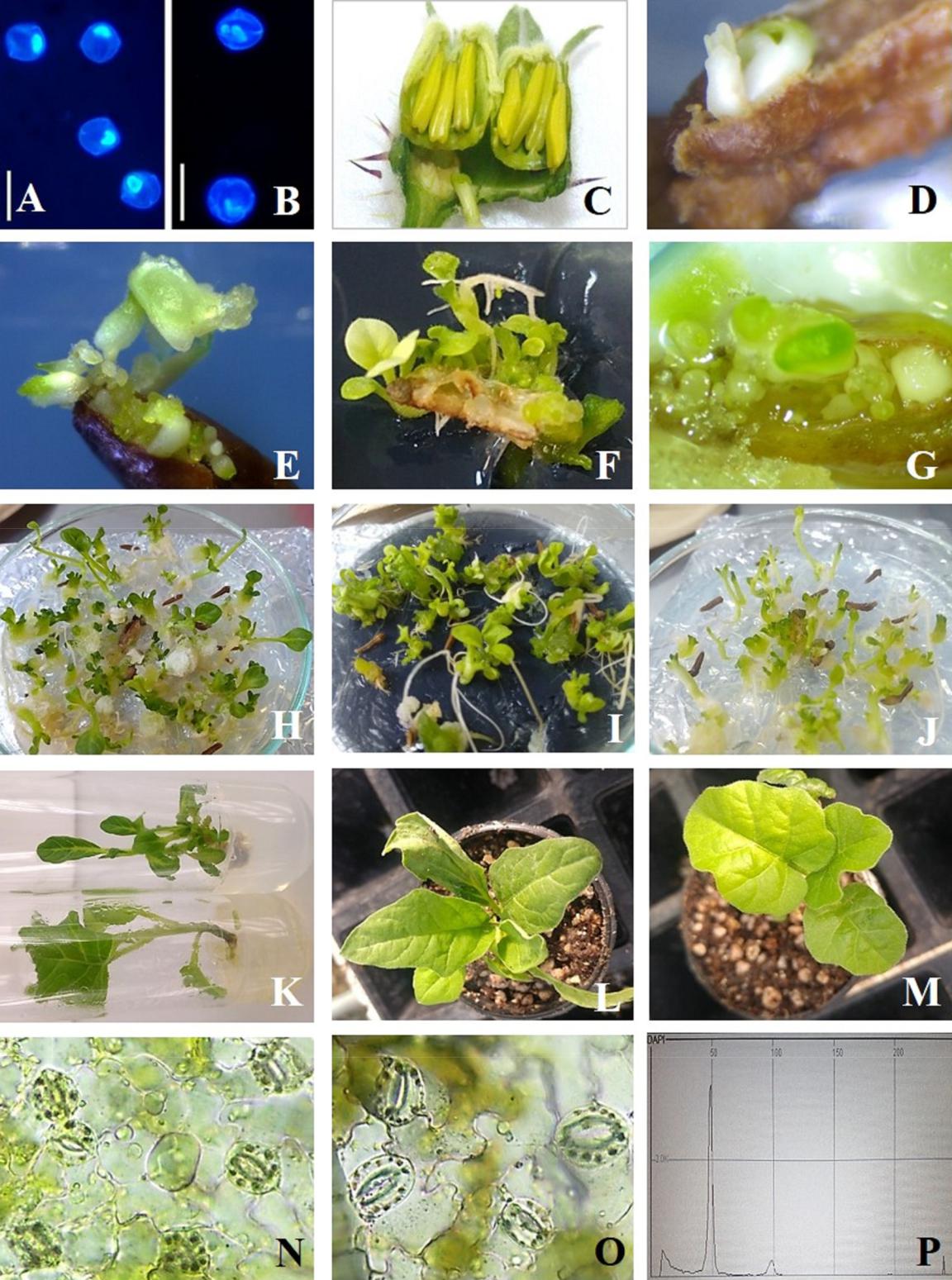
is 45.3 % while the acclimatization success rate is 90.1 %. The total yields of embryo, *in vitro* regenerated plantlet and acclimatized plants obtained from the anthers of ‘A117’ were 2669, 1267 and 1150, re-spectively. The same alignment for ‘Anamur’ was; 875, 337 and 295, respectively.

*3.2.2. Liquid anther cultures*

The effects of the factors consisting of genotype (‘A117’ and ‘Anamur’), induction (12 Mod-C) and differentiation (corresponding Mod-R and original DDV-R) medium on embryo and *in vitro* plantlet yields were researched also in liquid culture conditions in autumn. As mentioned in the method section, the number of induction media tested was reduced from 24 to 12 as AC use was excluded in liquid cultures. According to variance analysis of liquid cultures (data not shown), none of the independent factors had a significant effect on either yield. However, ‘A117’ between genotypes, the aLC12 medium among in-duction media and the original DDV-R medium between differentiation media was found to be better in promoting embryo formation. Microspore embryogenesis performances of both genotypes cultured in liquid cultures were much lower than solid ones in general. Both gen-otypes responded only to aLC10, aLC11 and aLC12 media (Table 1). Among them, aLC12 medium was the best to induce embryo formation. The anthers of ‘A117’ formed embryos only in the aLC12 (42 embryos/

8

*G.E. Vural and E. Ari* *Scientia Horticulturae 272 (2020) 109472*



**Fig. 5.** The stages of DH plant regeneration from anther cultures of ‘A117’ and ‘Anamur’ F1 eggplant cultivars: The late uninucleated (A), and early binucleatedmicrospore stages determined by DAPI (B), the anther morphology used for the cultures (C), the embryos coming out of an ‘Anamur’ anther cultured in the solid sSC5

* sR5 medium combination in spring (D), a very regenerative ‘A117’ anther cultured in a solid sSC24 + DDV-R medium combination in autumn (E), another regenerative ‘A117’ anther, in which a large number of embryos developed further growth, cultured in a solid sSC24 + corresponding Mod-R (aSR24) medium combination in autumn (F), the embryos at different stages occurring in an ‘A117’ anther cultured in a liquid aLC12 + liquid DDV medium combination in autumn (G), advanced growth of the embryos occurred in ‘A117’ anthers cultured in the comination of a solid aSC24 + DDV-R medium (H), and a solid aSC24 + corresponding Mod-R (aSR24) medium in autumn (I), advanced growth of the embryos occurred in ‘Anamur’ anthers cultured in the comination of a solid aSC24 + DDV-R medium (J), *in vitro* development of a haploid (above) and a spontaneous DH plant (below), according to the morphological observation (K), following the aclimatization stage, *in vivo* development of a haploid plant (L), and a spontaneous DH plant, based on the morphological observation (M), numbers of the chlor-oplasts in the stomata guard cells of a haploid (N), and a spontaneous DH plant (O), flow cytometer peaks of a haploid *ex vitro* plant of ‘A117 (P). *Bars* (A–B): 30 μm.

9

*G.E. Vural and E. Ari*

100 anthers) and aLC10 media (2 embryos/100 anthers) whereas those of ‘Anamur’ formed embryo only in the aLC11 medium (2 embryos/100 anthers). The common factor in these media was that they had a con-tent of 90 g/L maltose and AgNO3. The aLC10 and aLC12 media con-tained 10 mg/L AgNO3 while the aLC11 medium had 5 mg/L AgNO3. As the control medium of liquid cultures, aLC1 (the liquid form of original DDV medium) did not produce any embryogenic response in either genotype. The proper differentiation medium was DDV-R for ‘A117’ and Mod-R for ‘Anamur’ as in the solid cultures of the autumn season. The highest (42 embryos/100 anthers) embryo production took place in the anthers of ‘A117’ cultured first in the aLC12 induction medium, then in the DDV-R differentiation medium (Fig. 5). The single *in vitro* plantlet formation (4 plantlets/100 anthers) occurred from the embryos of ‘A117’ cultured in the same medium combination. A total of 46 em-bryos, four *in vitro* plantlets and two acclimatized plants (from ‘A117’) were obtained from liquid cultures of the autumn season.

*3.3. Ploidy analyses*

In the ploidy analyses based on morphological observations, in some cases, it was possible to foresee the haploid and spontaneous DH re-generants in *in vitro* conditions as well as *in vivo* conditions. In general, the haploid regenerants had slower growth tendency and narrower and smaller leaves, and their leaf veins were closer each other than those of DH regenerants. However, DH regenerants had larger leaves and pollen, and grew and rooted much faster just like normal diploid plants (Fig. 5). In the morphological observations of the total 1492 plants, the ratios of haploid, spontaneous DH and uncertain plants were found to be ap-proximately 47 %, 45 % and 8 %, respectively.

In the chloroplast countings, the chloroplast number of haploid plants had almost half the number of chloroplasts in DH plants. The number of the chloroplasts per guard cell pair was about 6–8 in haploid and 12–14 in spontaneous DH plants (Fig. 5), respectively. The ratios of haploid, DH and uncertain plants were detected as 50 %, 40 % and 10 %, respectively, among the 120 plants.

In the flow cytometry analysis of the 100′A117′ *ex vitro* regenerated plants, the ratios of haploid (Fig. 5), spontaneous DH, and mixoploid plants were determined to be 49 %, 44 % and 7 %, respectively. The same alignment for the morphological observation results of the same plants are 51 %, 43 % and 6 %, respectively. It is therefore possible to say that the cytometric analysis results of 100′A117′ plants highly confirmed the results obtained from the morphological observations.

DH plant regeneration stages of eggplant anthers cultured in this study are presented in Fig. 5A – P.

**4. Discussion**

DH plants need to be produced in large numbers in order to be used effectively in breeding studies. Anther culture is the most widely used haploid technique to obtain DH plants in eggplant. The two-stage protocol, known as the DDV protocol, consisting of induction and dif-ferentiation culture media is generally accepted to be reliable and re-producible for eggplant anther cultures. Thus, the DDV protocol or its minor modifications remain the most widely used protocols since 1982 (Segui-Simarro et al., 2011; Rotino, 2016). However, as indicated by Rotino (2016), the existing anther culture protocols need to be opti-mized especially for different genotypes that respond poorly to micro-spore embryogenesis. In addition, it has been proposed to focus on the development of suitable media composition to overcome abnormal embryo formation and low embryo germination rates in eggplant ([Salas](#page14) [et al., 2011](#page14); [Segui-Simarro et al., 2011](#page14)).

We did a very comprehensive study to optimize the DDV protocol in terms of both induction and differentiation media, and thereby to in-crease embryo and *in vitro* plantlet yields of eggplant anther cultures. In doing so, we investigated the effects of genotype, the growth season of donor plants, and the type of anther culture, as well as induction and

*Scientia Horticulturae 272 (2020) 109472*

differentiation media on embryo and *in vitro* plantlet yields.

*4.1. Effect of genotype*

Although there was no statistically significant independent geno-typic effect on embryo and *in vitro* plantlet yield in the spring season, the effect of genotype was significant (*p*< 0.001) in autumn. Genotypic differences have also been mentioned in other eggplant an-ther culture studies (Tuberosa et al., 1987; Karakullukcu, 1991; [Rotino,](#page14) [1996](#page14); Salas et al., 2011, Basay and Ellialtıoglu, 2013; Rivas-Sendra et al., 2017). Among our genotypes, ‘A117’ gave the highest average embryo and *in vitro* plantlet yields in both seasons, especially in autumn with yields of 320 embryos and 200 plantlets/100 anthers. From the anthers of the same genotype, Ozdemir-Celik (2018) using the DDV protocol obtained 42.68 % embryo and 16.81 % plantlet. As for ‘Anamur’ F1, we found the highest embryo and *in vitro* plantlet yields to be 100 embryos and 67 plantlets/100 anthers. In the study of [Gebologlu](#page13) et al. (2017), who specified ‘Anamur’ F1 to have a low androgenic performance, the highest embryoid yields of ‘Anamur’ appears to be 9.73 per 10 anthers, but there is no information about *in vitro* plantlet formation. In the other eggplant anther culture studies using different commercial cultivars from us, the highest yields were reported to be 53 embryos/100 anthers in Cristal F1 by Salas et al. (2012), 60.9 embryos and 5.8 plantlets/100 anthers in Ecavi F1 by Salas et al. (2011) and 146.46 embryos/100 anthers from Bandera F1 by Rivas-Sendra et al. [(2017)](#page14). In the last study, the embryo yield of a highly responsive DH line, DH36, improved by Bandera F1 was reported to be 237.50 em-bryos/100 anthers. Apparently, as far as we know, the highest embryo and *in vitro* plantlet yields (320 embryos and 200 plantlets/100 anthers) until today have been obtained from ‘A117’ F1 in the present study.

*4.2. Effect of growth season of donor plants*

The androgenic performances of the genotypes were much higher in autumn than in spring. After excluding the yield of ‘Darko’ that was not used in autumn, the highest average embryo yields of ‘A117’ and ‘Anamur’ were 11 and 8 embryos/100 anthers, respectively, in spring. These yields increased to 134 and 72 embryos/100 anthers, respec-tively, for the same genotypes in the related common culture media of autumn. In fact, the highest embryo yield, mentioned above for ‘A117’ in autumn, reached 320 embryos/100 anthers, through the aSC24 medium which was not used in spring. Seasonal variations in embryo yield of eggplant anther cultures have been reported previously. Tuberosa et al. (1987) determined the most responsive anthers were from mid-September to mid-October during the period of July–October (in the Northern Hemisphere). Also, Rotino (2016) working in Medi-terranean climate conditions achieved higher androgenetic frequencies in cooler months (noted as an unpublished result) and stated that spring and autumn were the best periods for eggplant anther cultures. As in-dicated by Dunwell (1985), growth conditions of donors such as tem-perature, light intensity, and lighting period affect microspore devel-opment, and therefore embryo yield. According to our culture results, climate conditions in autumn mentioned in the method section are optimum and thus autumn is the superior time to obtain much higher yields of embryo and *in vitro* plantlet in eggplant anther culture in Antalya under Mediterranean conditions.

*4.3. Effect of anther culture type*

Among the anther culture types, solid cultures were the best in both seasons. No response was received from double-layered (shed-micro-spore) cultures in the spring season. All anthers of this culture type turned brown or black at the end of the first 8-days of incubation and lost their vitality, and therefore this culture type was excluded in au-tumn. This negative result could be due to the misuse or high level use of AC in an inappropriate layer since we added AC directly to both

10

*G.E. Vural and E. Ari* *Scientia Horticulturae 272 (2020) 109472*

layers of double-layered cultures at the same concentration (1 g/L). According to Gland et al. (1988), AC should be used together with agarose for isolated microspores in liquid cultures, since suspended charcoal without agarose adheres to microspores and prevent embry-ogenesis. The negative effect of the direct use of AC without agarose as in our study was previously reported, but in various isolated microspore culture studies (Kott et al., 1988; Prem et al., 2005; Takahashi et al., [2012](#page14)). Seemingly, this type of AC use also damages anthers in liquid cultures, despite their thick walls. However, we still consider that the shed-microspore culture technique may be improved for eggplant with the optimization of suitable AC use at different concentrations and in the proper layer.

As for the liquid anther culture type, we obtained an embryo yield of up to 42 embryos/100 anthers in ‘A117’ anthers cultured first in an aLC12-encoded liquid medium (with 90 g/L maltose +10 mg/L AgNO3), and then in liquid the DDV-R medium only in autumn. Although liquid cultures had lower efficacy on embryogenesis than solid ones, as far as we know, this is the first study on liquid eggplant anther cultures and has the potential to be developed. The positive effect of maltose on liquid anther cultures was revealed previously in wheat (Fadel and Wenzel, 1990), timothy (Guo et al., 1999) and highly recalcitrant rice anther cultures (Lentini et al. (1995), especially with the combined use of maltose and AgNO3 in the last study. Therefore, the efficiency of liquid anther cultures can be improved by the optimization of maltose and AgNO3 concentrations and the addition of AC with agarose.

*4.4. Effects of the optimization chemicals used in induction and differentiation media*

The original DDV protocol has been used in almost all eggplant anther culture studies to date. It was used also in this study as the control and it was optimized with additives for both the induction and differentiation stages. While no embryos occurred in the control medium of ‘A117’ and ‘Anamur’ in spring (Fig. 1), the embryo and *in* *vitro* plantlet yields of the control medium in autumn were 134 embryosand 63 plantlets/100 anthers for ‘A117’ and 24 embryos and 12 plantlets/100 anthers for ‘Anamur’ as the control results of the geno-type x induction medium x differentiation medium interaction (Table 2). This result shows again the supremacy of autumn for high androgenic performance of ‘A117’ and ‘Anamur’.

The effects of the induction medium alone and also the interaction of genotype x induction medium on the yields of embryo and *in vitro* plantlets were found significant (*p* < 0.001) in both seasons. However, each of the optimization chemicals and their interactions with each other had a different influence on the yields. In order to better reflect their single, dual, and triple effects, they will be discussed in particular through Fig. 4 with six sub-graphs, as the result of the interaction effect of the genotype x induction medium in the autumn season (*p* < 0.001), regardless of the effect of the differentiation medium.

Regarding only the effect of carbohydrate sources, the efficacies of different concentrations of maltose (30, 60 and 90 g/L) as well as su-crose (120 g/L) will be evaluated first. Regarding the effect of maltose alone, embryo yields of both ‘A117’ and ‘Anamur’ gradually increased in general with the increasing amount of maltose in the medium (Fig. 4A). Lower concentrations of maltose (30 and 60 g/L) containing media were not as effective as the medium containing either sucrose or a high concentration of maltose to increase embryo yields of both genotypes. The highly concentrated (90 g/L) maltose medium (aSC19) is 1.5 and 2 times more effective than sucrose containing the control medium for ‘A117’ and ‘Anamur’, respectively. The superiority of maltose to sucrose was also indicated in the anther cultures of other Solanaceae species such as petunia (Raquin, 1983) and pepper (Dolcet-Sanjuan et al., 1997;). However, the use of maltose in eggplant anther culture is very rare. Gemes Juhasz et al. (2006) using the DDV protocol,

but exchanging 120 g/L sucrose with 120 g/L maltose, noted without any details that maltose increased the ratio of responding anthers and regeneration. Likewise, as an unpublished result, Salas et al. (2011) stated they observed a higher embryogenic response from only ‘Ban-dera’ genotype cultured in a medium with maltose, again without de-tails. According to Bal et al. (2009), the use of maltose in isolated mi-crospore culture of eggplant stimulates symmetrical divisions of the microspores, and it can be used instead of sucrose in eggplant especially for direct embryogenesis. As known, carbohydrates are used both as a carbon source and as an osmotic regulator in the nutrient media. Both functions have a critical role in embryo formation. As the most used carbohydrate, sucrose is very rapidly hydrolyzed and raises the os-molality of the culture medium (Last and Brettell, 1990; Lentini et al., [1995](#page14)). In contrast, maltose is slowly hydrolyzed (Roberts-Oehlschlager et al., 1990) and the changes in osmotic pressure of the maltose con-taining media are unimportant (Goralski et al., 2002). Thus, maltose provides a more stable culture medium. Scott et al. (1995) explained the cause of high maltose efficiency with its slower metabolism and the ability to provide adequate oxygen to cultures compared to sucrose. Thereby, the cells survive longer in the culture. Other advantages of maltose are its contribution to green plant production (Fadel and Wenzel, 1990; Guo et al., 1999) and direct regeneration (Finnie et al., [1989](#page13)). We also observed the positive effects of maltose in terms of higher anther viability, direct embryogenesis, and healthy regenera-tion.

As for the efficiencies of the use of sucrose or maltose together with two additives (AgNO3 or/and AC), the effect of using AgNO3 will be mentioned first. WhenFig. 4A, Fig. 4B and Fig. 4C were compared, it is obvious that the inclusion of 5 mg/L (aSC2) or 10 mg/L (aSC3) AgNO3 into the sucrose (120 g/L) containing medium caused a serious decrease in embryo yields of both genotypes compared to the control medium (aSC1). AgNO3 use made sense only in the most highly concentrated maltose media. Also, the use of 5 mg/L AgNO3 + 90 g/L maltose was more effective than 10 mg/L AgNO3 + 90 g/L, but did not have a very striking result on the yield. However, AgNO3 has been used for a long time in anther cultures of many species because of its inhibitory effect on ethylene action in tissues (Beyer, 1976). AgNO3 has a promoting effect on microspore embryogenesis by lowering ethylene production and preventing the inhibitory effect of excessive endogenous ethylene (Dunwell, 1979; Biddington et al., 1988; Cho and Kasha, 1989). There are even reports saying that no embryo formed unless AgNO3 was added to the culture medium as in the anther culture studies of Brussel sprouts (Biddigton et al., 1988), *Brassica oleracea* (Dias and Martins, [1999](#page13)), *B. juncea* (Malik et al., 2001; Prem et al., 2005), Kahramanmaras pepper (Comlekcioglu et al., 2001). Also, AgNO3 absorbs deleterious metabolic substances produced during embryonic development, pro-motes microspore-derived embryo (Zhang et al., 1997; Wu et al., 2006; Wang et al., 2011) and shoot (Hyde and Phillips, 1996; Panigrahi et al., [2018](#page14);) regeneration and a higher survival rate of *ex vitro* plantlets (Giridhar et al., 2001). However, AgNO3 has been used in sucrose-containing media in most studies and its use with maltose is quite rare in literature. Lentini et al. (1995) stated in such an example that the anthers cultured in a medium containing maltose + AgNO3 may be less influenced by ethylene. Because of this, while sucrose increases ethy-lene production in wounded tissues (Meir et al., 1984), combination of maltose and AgNO3 ensures less exposure of anthers to ethylene. In our study, combined use of maltose + AgNO3 relatively improved embryo formation only in ‘Anamur’ anthers cultured in aSC20 medium (with 90 g/L maltose +5 mg/L AgNO3). As a result, the use of AgNO3 to-gether with maltose has an effect dependent on genotype and the concentrations of both maltose and AgNO3 in eggplant anther cultures.

Regarding the effect of using AC with maltose or sucrose, according to Fig. 4A and Fig. 4D, the use of 0.1 % AC (1 g/L) caused a 5–6 fold decrease in embryo yields of both genotypes cultured especially in a highly concentrated maltose (90 g/L) medium (aSC22) compared to other maltose containing media. In a sucrose containing medium with

11

*G.E. Vural and E. Ari*

AC (aSC4), a 12.3-fold sharp decline occurred in ‘A117' but a slight decrease in ‘Anamur’ compared to the control DDV medium (aSC1). Therefore, it is clear that the addition of 0.1 % 0.1 % AC to both sucrose and maltose containing media without AgNO3, negatively affected embryo formation in both genotypes. However, AC, as an undefined additive, has been included in many tissue culture systems including anther cultures for a long time. It has several advantages for the cul-tures such as absorbing some inhibitory compounds released particu-larly from newly cultured wounded tissues or present in culture media, agar or autoclaving products, a promoting effect on morphogenesis, especially embryogenesis (George, 1993), trapping some gases like ethylene and carbon dioxide (Ernst, 1974; Johansson, 1983), pre-venting undesired callus growth, and encouraging better development of the embryos, and thus their easier transformation into whole plants (Gland, 1998). According to George (1993), AC improves or regulates *in* *vitro* plant growth in some circumstances although it is not a PGR. Theembryogenesis stimulating effects of AC were reported by [Johansson](#page13) et al. (1982) as some substance(s) released by AC itself. However, AC also has an adsorption effect on phenolics, produced by anther walls, and certain media ingredients especially PGRs and organic nutrients. Because of its absorption and adsorption effects, AC changes the culture medium in a number of ways (Dunwell, 1991). When used at high le-vels, AC makes the beneficial compounds unusable for the culture ([Dias,](#page13) [1999](#page13)). Thus, use of AC may be useful or harmful for *in vitro* cultures, depending on several factors including the culture medium, species, and tissues used (Pan and Staden, 1999). There are different reports on the positive or negative influences of this compound saying that AC increased the number of embryos and plantlets per anther and ac-celerated the regeneration of plantlets from anther ([Anagnostakis,](#page13) [1974](#page13); Bajaj, 1983), or caused anthers to darken and lose their vitality (Karakullukcu, 1991; Ari, 2006). The efficacy of AC was also tested in a limited number of eggplant anther culture studies. Karakullukcu (1991) and Tipirdamaz and Ellialtioglu (1998) reported a detrimental or ne-gative effect of 1 % AC on anther vitality and embryo induction in a sucrose containing DDV medium. AC use in maltose-containing media is generally less common among androgenesis studies, but it appears more effective on both embryogenesis induction and subsequent embryo differentiation and plant regeneration in several studies (Dolcet-Sanjuan et al., 1997; Supena et al., 2006). In our culture conditions, the interaction of AC x maltose as well as AC x sucrose caused a negative effect on embryo yields of both ‘A117' and ‘Anamur’. However, the embryo yields of both genotypes increased dramatically when AgNO3 was added to both combinations ([Fig. 4](#page7)E - F).

Regarding the triple interaction of the carbohydrate source x AC x AgNO3, the addition of 1 g/L AC + 10 mg/L AgNO3 to sucrose or maltose containing almost all media generally had a clear positive ef-fect on embryo yields of both genotypes (Fig. 4F). In particular, an aSC24-encoded Mod-C medium (with 90 g/L maltose +10 mg/L AgNO3 + 1 g/L AC) created a significant triple synergistic effect on embryo yield. Especially the anthers of ‘A117’ cultured in aSC24 were so regenerative that many small embryos were detached from the an-thers and new embryos continued to emerge from the same anthers. The embryo yield of ‘A117’ in aSC24 (290 embryos/100 anthers, as independent from the effect of differentiation medium) greatly sur-passed the yields of all other 23 Mod-C media in Fig. 4 including the control medium (aSC1), which is the original DDV-C medium, (*p* < 0.001). The same circumstance applies to ‘Anamur’ with one exception. ‘Anamur’ gave the highest embryo yield first in an aSC20 medium (with 90 g/L maltose + 5 mg/L AgNO3) (74 embryos/100 anthers), then in an aSC24 medium (62 embryos/100 anthers). On the other hand, the embryo-forming effect of the combined use of 10 mg/L AgNO3 + 1 g/L AC with sucrose was far behind than those with a higher concentration of maltose. Because of this, the embryo yield of ‘A117’ obtained from aSC24 is 5.2-fold higher than from aSC6 (with 120 g/L sucrose + 10 mg/L AgNO3 + 1 g/L AC) and also 3.9 times higher than from the control medium (aSC1). In the ‘Anamur’ genotype, aSC24 formed 1.2

*Scientia Horticulturae 272 (2020) 109472*

and 3.9-fold higher embryo numbers than aSC6 and aSC1, respectively. As an interesting result, the addition of 1 g/L AC almost completely prevented callus formation in all media with or without AgNO3, par-ticularly in maltose-containing media (data not shown). A similar result was previously demonstrated in *Anemone coronaria* anther cultures ([Ari,](#page13) [2006](#page13)) where the carbohydrate source was sucrose (2 %) and the AC ratio was 1 %. Although the information is lacking on the positive effect of the combined use of AgNO3 + AC in maltose-containing media, it is possible to come across different reports about those in sucrose-con-taining medium, especially in anther cultures of pepper ([Comlekcioglu](#page13) et al., 2001; Buyukalaca et al., 2004; Taskin et al., 2011) and isolated microspore cultures of the Brassica species. For instance, Prem et al. [(2008)](#page14) obtained a fourfold increase in the embryo yield of *B. juncea* when they added AC to an AgNO3 + sucrose medium. In another ex-ample, Na et al. (2011) notified more than a 2-fold increase in embryo yield of broccoli when AgNO3 was added to an AC + sucrose medium.

The effects of Mod-C media discussed so far. While the induction medium has a critical role to initiate microspore embryogenesis in eggplant anther cultures, the differentiation medium is also very im-portant for the further development of embryos. Hence, it is re-commended to test alternative induction and regeneration media si-multaneously, in particular for novel genotypes or segregating progenies (Rotino, 2016). In terms of the effect of differentiation media especially in autumn, the effects of Mod-R or the original DDV-R medium did not cause a statistically significant difference on the yields of embryos and *in vitro* plantlet yields. However, while Mod-R media produced slightly more embryos and *in vitro* plantlets in general than the DDV-R medium in ‘A117’, the situation was the opposite for ‘Anamur’. The highest embryo and *in vitro* plantlet yields of the present study were recorded to be 320 embryos and 200 plantlets/100 anthers in ‘A117’ cultured in aSC24 + original DDV-R media. In ‘Anamur’, the highest embryo yield (100 embryos/100 anthers) was obtained from the medium combination of aSC20 + DDV-R while the highest *in vitro* plantlet yield (67 plantlets/100 anthers) formed in the combination of aSC24 + Mod-R (Table 2). As seen, embryo and *in vitro* plantlet yields of ‘both genotypes varied in different induction-differentiation media, and the androgenic performance of ‘A117’ was much better than ‘Anamur’ in the whole study, particularly in autumn. This performance variation might be explained mainly by genotypic effect and also other factors such as anther wall thickness, ethylene production and the phytochemical (secondary metabolite) content of the genotypes in-cluding alkaloids, phenolics *etc.*

**5. Conclusions**

The single effect of maltose use without any additives on micro-spore-derived embryo yield of eggplant anther cultures was consider-able only in the most highly concentrated maltose containing medium (90 g/L). While the effects of maltose + AgNO3 combinations (with 5 mg/L AgNO3 or 10 mg/L AgNO3) were not notable, the effect of the maltose + AC combination was negative. However, the triple effect of the maltose + AgNO3 + AC combination was found remarkably sy-nergistic especially in the presence of high concentrations of both maltose (90 g/L) and AgNO3 (10 mg/L) in addition to AC (0.1 %) for ‘A117’ anthers cultured in the autumn season. When considering the performance of ‘A117’ in the spring season, in which high concentra-tions of maltose was not used, it is possible to say that this synergistic effect depends on the season, genotype, and concentrations of optimi-zation chemicals.

All the positive effects of maltose, AgN03 and AC, mentioned above in detail, were clearly revealed in the present study when all three chemicals were used together. We observed their triple positive sy-nergistic effects on longer vitality of anthers, embryo quality, undesired callus and abnormal embryo formation, direct embryogenesis, faster regeneration, and finally higher embryo and *in vitro* plantlet yields. These result are supported by our high embryo yield and high rates of

12

*G.E. Vural and E. Ari*

transformation of embryos to *in vitro* plantlets (45.3 %) and acclima-tization (90.1 %) especially in the autumn season. As a result, we im-proved an extremely efficient modified induction medium consisting of 90 g/L maltose +10 mg/L AgN03 + 1 g/L AC in addition to macro-micro salts, vitamins and PGRs of the original DDV medium. Through aSC24-encoded this Mod-C medium, we achieved the highest embryo and *in vitro* plantlet yields of up to 320 embryos/100 anthers and 200 plantlets/100 anthers with the help of the DDV-R differentiation medium from ‘A117’ anthers cultured in autumn. Also, embryo yields of both genotypes cultured in aSC24 were 3.9 times higher than the ori-ginal DDV medium. In fact, It may be interesting to test the use of these modified triple additives with other basic culture media instead of the DDV media in the future. Finally, as far as we know, embryos (up to 42 embryos/100 anthers) were obtained for the first time in a liquid eggplant anther culture, which might be useful for direct embryogen-esis in isolated microspore cultures.

**CRediT authorship contribution statement**

**Gulsun Elif Vural:** Investigation, Resources, Formal analysis. **Esin Ari:** Conceptualization, Methodology, Visualization, Supervision,Formal analysis, Writing - review & editing.

**Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influ-ence the work reported in this paper.

**Acknowledgement**

This study was carried out within the scope of master thesis of Gulsun Elif VURAL in Akdeniz Univ. Inst. of Science, Antalya, Turkey. Authors thanks to Antalya Tarim Co. R & D Center for supporting this study and to Mr. Nicholas Lambourn for English editing.

**Appendix A. Supplementary data**

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.scienta.2020.109472>.

**References**

Alpsoy, H.C., Seniz, V., 2007. Researches on the *in vitro* androgenesis and obtaining [haploid plants in some eggplant genotypes. Acta Hortic. (ISHS) 729, 137–141](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0005).

[Anagnostakis, S.L., 1974. Haploid plants from tobacco- Enhancement with charcoal.](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0010)

[Planta 115, 281–283](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0010).

Ari, E., 2006. Anther Culture Studies on *Anemone coronaria* Var. *Coccinea* Native to Turkey. Cukurova Univ., Institute of Science, Adana, pp. 169 Doctoral dissertation in [Turkish](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0015).

Ari, E., Bedir, H., Yildirim, S., Yildirim, T., 2016a. Androgenic responses of 64 ornamental pepper (*Capsicum annuum* L.) genotypes to shed-microspore culture in the autumn [season. Turk. J. Biol. 40 (3), 706–717](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0020).

Ari, E., Yildirim, T., Mutlu, N., Buyukalaca, S., Gokmen, U., Akman, E., 2016b. Comparison of different androgenesis protocols for doubled haploid plant production [in ornamental pepper (*Capsicum annuum* L.). Turk. J. Biol. 40 (4), 944–954](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0025).

Bajaj, Y.P.S., 1983. *In vitro* production of haploids. In: In: Evans, D.A., Hharp, W.R.S., Ammirato, P.V., Yamada, Y. (Eds.), Handbook of Plant Cell Culture Techniques of [Propagation and Breeding 1. Collier Macmillan Publishers, London, pp. 228–287](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0030).

Bal, U., Ellialtioglu, S., Abak, K., 2009. Induction of symmetrical nucleus division and multi-nucleate structureGaos in microspores of eggplant (*Solanum melongena* L.) [cultured *in vitro*. Sci. Agric. 66 (4), 535–539](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0035).

Basay, S., Ellialtıoglu, S.S., 2013. Effect of genotypical factors on the effectiveness of [anther culture in eggplant (*Solanum melongena* L.). Turk. J. Biol. 37 (4), 499–505](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0040).

Beyer, E.M., 1976. A potent inhibitor of ethylene action in plants. Plant Physiolog 58 (3), [268–271](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0045).

Biddington, N.L., Sutherland, R.A., Robinson, H.T., 1988. Silver nitrate increases embryo [production in anther culture of Brussels sprouts. Ann. Bot. 62 (2), 181–185](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0050).

Bravo, L., 1998. Polyphenols: chemistry, dietary sources, metabolism, and nutritional [significance. Nutr. Rev. 56 (11),](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0055) 317–333.

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*

*Scientia Horticulturae 272 (2020) 109472*

[*annuum* L.) haploid embryos via anther culture. Eur. J. Hortic. Sci. 69, 206–209](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0060).

[Cao, G., Sofic, E., Prior, R.L., 1996. Antioxidant capacity of tea and common vegetables.](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0065) J.

[Agric. Food Chem. 44 (11), 3426–3431](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0065).

Chambonnet, D., 1985. Culture D’antheres *in vitro* chez trois *Solanaceaes* maraicheres. Le Piment (*Capsicum Annuum* L.), l’aubergine (*Solanum Melongena* L.), La Tomato (*Lycopersicon Esculentum* Mill.) Et Obtention De Plantes Haploides. Academie de [Montpellier, In French, pp. 90 Doct. dissert.](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0070)

Cho, U.H., Kasha, K.J., 1989. Ethylene production and embyogenesis from anther cultures [of barley (*Hordeum vulgare*). Plant Cell Rep. 8 (7), 415–417](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0075).

Comlekcioglu, N., Buyukalaca, S., Abak, K., 2001. Effect of silver nitrate on haploid embryo induction by anther culture in pepper (*Capsicum annuum* L.). In: In *XIth* EUCARPIA Meeting on Genetics and Breeding of Capsicum and Eggplant. Antalya, [Turkey. pp. 133–136](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0080).

Corral-Martinez, P., Segui-Simarro, J.M., 2012. Efficient production of callus-derived doubled haploids through isolated microspore culture in eggplant (*Solanum melon-*[*gena* L.). Euphytica 187 (1), 47–61](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0085).

Custers, J.B.M., 2003. Microspore Culture in Rapeseed (*Brassica Napus* L.). In Doubled [Haploid Production in Crop Plants. Springer, Dordrecht, pp. 185–193](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0090).

Dias, J.C., 1999. Effect of activated charcoal on *Brassica oleracea* microspore culture [embryogenesis. Euphytica 108 (1), 65–69](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0095).

Dias, J.S., Martins, M.G., 1999. Effect of silver nitrate on anther culture embryo pro-[duction of different *Brassica oleracea* morphotypes. Sci. Hortic. 82 (3-4), 299–307](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0100).

[Daunay, M.C., Dalmon, A., Lester, R.N., 1999. In: Nee, M., Symon, D.E., Lester, R.N.,](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0105)

[Jessop, J.P. (Eds.), Management of a Collection of Solanum Species for Eggplant](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0105)

[(*Solanum melongena*) Breeding Purposes. Royal Botanic Gardens, Kew, pp. 369–383](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0105)

[Solanaceae IV edited by](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0105).

Dolcet-Sanjuan, R., Claveria, E., Huerta, A., 1997. Androgenesis in *Capsicum annuum* L.—effects of carbohydrate and carbon dioxide enrichment. J. Am. Soc. Hortic. Sci. [122 (4), 468–475](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0110).

[Dumas de Vaulx, R., Chambonnet, D., 1982. Culture *in vitro* d’anthères d’aubergine](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0115)

[(*Solanum melongena* L.): stimulation de la production de plantes au moyen de trai-](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0115)

[tements à+ 35 C associés à de faibles teneurs en substances de croissance. Agronomie](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0115)

[2 (10), 983–988](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0115).

Dunwell, J.M., 1979. Anther culture in *Nicotiana tabacum*: the role of the culture vessel [atmosphere in pollen embryo induction and growth. J. Exp. Bot. 30 (3), 419–428](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0120).

[Dunwell, J.M., 1985. Anther and ovary culture. In Cereal Tissue and Cell Culture.](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0125)

[Springer, pp. 1–44](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0125).

Dunwell, J.W., 1991. Haploid cell cultures. In: Dixon, R.A. (Ed.), ‘Plant Cell Culture: a [Practical Approach’. IRC Pres, Oxford, Washington D.C, pp. 21–36](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0130).

Ellialtıoglu, S.S., Tipirdamaz, R., 1999. Patlican Anter Kulturunde Absizik Asit Miktarini [Azaltici Uygulamalarin Androgenetik Embriyo olusumuna etkisi. Kukem Dergisi. 24](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0135)

[(1), 23–32](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0135).

Ernst, R., 1974. Use of activated charcoal in asymbiotic seedling culture of [Paphiopedilum. Am Orc Soc Bull. 36, 386–394](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0140).

Fadel, F., Wenzel, G., 1990. Medium‐genotype‐interaction on androgenetic haploid pro-[duction in wheat. Plant Breed. 105 (4), 278–282](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0145).

[FAOSTAT, 2019. FAO Statistical Database (accessed 10.05.2019).](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0150) .

Finnie, S.J., Powll, W., Dyer, A.F., 1989. The effect of carbohydrate composition and concentration on anther culture response in barley (*Hordeum vulgare L*.). Plant Breed. [103 (2), 110–118](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0155).

Gebologlu, N., Boncukcu, S.D., Durna, P., Bayram, M., 2017. Patlıcanda seker, bal ve buyume duzenleyicilerin anter kulturunde embriyoid olusumuna etkisi. Akad. Ziraat [Derg. 6, 275–280](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0160).

Gemes Juhasz, A., Vencel, G., Sagi, Z.S., Gajdos, L., Kristof, Z., Vagi, P., Zatyko, L., 2006. Production of doubled haploid breeding lines in case of paprika, eggplant, cucumber, [zucchini and onion. Acta Hortic. 725, 845–854](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0165).

George, E.F., 1993. Plant Propagation by Tissue Culture. Part 1: the Technology, ed. 2. . Giridhar, P., Reddy, B.O., Ravishankar, G.A., 2001. Silver nitrate influences *in vitro* shoot [multiplication and root formation in *Vanilla planifolia* Andr. Curr. Sci. 81 (9),](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0175)

[1166–1170](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0175).

Gland, A., Lichter, R., Schweiger, H.G., 1988. Genetic and exogenous factors affecting embryogenesis in isolated microspore cultures of *Brassica napus* L. J. Plant Physiol. [132 (5), 613–617](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0180).

Goralski, G., Lafitte, C., Bouazza, L., Matthys-Rochon, E., Przywara, L., 2002. Influence of sugars on isolated microspore development in maize (*Zea mays* L.). Acta Biol Cra Ser [Bot 44, 203–212](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0185).

Guo, Y.D., Sewón, P., Pulli, S., 1999. Improved embryogenesis from anther culture and [plant regeneration in timothy. Plant Cell Tissue Organ Cult. 57 (2), 85–93](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0190).

Hyde, C., Phillips, G.C., 1996. Silver nitrate promotes shoot development and plant re-generation of chile pepper (*Capsicum annuum* L.) via organogenesis. In Vitro Cell. [Dev. Biol., Plant 32, 72–80](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0195).

Isouard, G., Raquin, C., Demarly, Y., 1979. Obtention de plantes haploides et diploides par culture *in vitro* d’anthères d’aubergine (*Solanum melongena* L.). C R Acad Sci Paris [288, 987–989](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0200).

[Johansson, L., 1983. Effects of activated charcoal in anther cultures. Physiol. Plant.](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0205) 59

[(3), 397–403](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0205).

Johansson, L., Andersson, B., Eriksson, T., 1982. Improvement of anther culture tech-nique: activated charcoal bound in agar medium in combination with liquid medium [and elevated CO2 concentration. Physiol. Plant. 54 (1), 24–30](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0210).

Kakizaki, Y., 1931. Hybrid vigor in eggplants and its practical utilization. Genetics 16, [1–25](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0215).

Karakullukcu, S., 1991. Investigations on Some Factors Inducingin Vitro Androgenesis and Haploid Plant Development in Different Eggplant Genotypes. Ankara University, [Institute of Science, Ankara, Turkey, pp. 136 Doctoral dissertation in Turkish](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0220).

Karakullukcu, S., Abak, K., 1993a. Studies on anther culture of eggplant. I. determination of the suitable bud stage. Turk. J. Agric. For. 17, 801–810.

13

*G.E. Vural and E. Ari*

Karakullukcu, S., Abak, K., 1993b. Studies on anther culture of eggplant. II.. Effects of [sugars and growth regulators. Turk. J. Agric. For. 17, 811–820](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0230).

Kaushik, P., Andujar, I., Vilanova, S., Plazas, M., Gramazio, P., Herraiz, F.J., Brar, N.S., Prohens, J., 2015. Breeding vegetables with increased content in bioactive phenolic [acids. Molecules 20 (10), 18464–18481](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0235).

Kott, L.S., Polsoni, L., Ellis, B., Beverdorf, W.D., 1988. Autotoxicity in isolated microspore [cultures of *Brassica napus*. Can. J. Bot. 66, 1665–1670](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0240).

Kuo, K.W., Hsu, S.H., Li, Y.P., Lin, W.L., Liu, L.F., Chang, L.C., Lin, C.C., Lin, C.N., Sheu, H.M., 2000. Anticancer activity evaluation of the Solanum glycoalkaloid sola-margine: triggering apoptosis in human hematoma cells. Biochem. Pharmacol. 60, [1865–1873](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0245).

Last, D.I., Brettell, R.I., 1990. Embryo yield in wheat anther culture is influenced by the [choice of sugar in the culture medium. Plant Cell Rep. 9 (1), 14–16](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0250).

Lentini, Z., Reyes, P., Martínez, C.P., Roca, W.M., 1995. Androgenesis of highly re-[calcitrant rice genotypes with maltose and silver nitrate. Plant Sci. 110 (1), 127–138](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0255).

Malik, M.R., Rangaswamy, N.S., Shivanna, K.R., 2001. Induction of microspore embryos in a CMS line of *Brassica juncea* and formation of the androgenic plantlets. Euphytica [120 (2), 195–203](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0260).

Meir, S., Philosoph-Hadas, S., Epstein, E., Aharoni, N., 1984. Role of Sucrose in the Metabolism of IAA-conjugates As Related to Ethylene Production by Tobacco Leaf [Discs. Ethylene. Springer, pp. 97–98](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0265).

Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassays with [tobacco tissue cultures. Physiol. Plant. 15, 473–497](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0270).

Na, H., Kwak, J.H., Chun, C., 2011. The effects of plant growth regulators, activated charcoal, and AgNO 3 on microspore derived embryo formation in broccoli (*Brassica* [*oleracea* L. var. *italica*). Hortic. Environ. Biotechnol. 52 (5), 524](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0275).

Ozdemir-Celik, B., 2018. The Effects Of Different Applicatıon To Induce Haploid Embryo And Plant Formation On Eggplant (*Solanum melongena*L.) Mıcrospore Culture. Akdeniz University, Institute of Science, Antalya, pp. 86 Doctoral dissertation in [Turkish](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0280).

Pan, M.J., Staden, J.V., 1999. Effect of activated charcoal, autoclaving and culture media [on sucrose hydrolysis. Plant Growth Regul. 29, 135–141](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0285).

Panigrahi, J., Dholu, P., Shah, T.J., Gantait, S., 2018. Silver nitrate-induced *in vitro* shoot multiplication and precocious flowering in *Catharanthus roseus* (L.) G. Don, a rich [source of terpenoid indole alkaloids. Plant Cell Tissue Organ Cult. 132 (3), 579–584](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0290).

Prem, D., Gupta, K., Agnihotri, A., 2005. Effect of various exogenous and endogenous factors on microspore embryogenesis in Indian mustard (*Brassica juncea* L., Czern & [Coss). In Vitro Cell. Dev. Biol., Plant 41, 266–273](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0295).

Prem, D., Gupta, K., Sarkar, G., Agnihotri, A., 2008. Activated charcoal induced high frequency microspore embryogenesis and efficient doubled haploid production in [Brassica juncea. Plant Cell Tissue Organ Cult. 93 (3), 269–282](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0300).

Raina, S.K., Iyer, R.D., 1973. Differentiation of diploid plants from pollen callus in anther [cultures of *Solanum melongena* L. Z Pflanzenzucht 70,](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0305) 275–280.

Raquin, C., 1983. Utilization of different sugars as carbon source for*in vitro* anther culture [of Petunia. Z Pflanzenphysiol 111 (5),](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0310) 453–457.

Research-Group-of-Haploid-Breeding, 1978. Induction of haploid plants of *Solanum mel-ongena*. In: Proceedings of the Symposium on Plant Tissue Culture. Peking, Science[Press. pp. 227–232](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0315).

Rivas-Sendra, A., Corral-Martinez, P., Camacho-Fernandez, C., Segui-Simarro, J.M., 2015. Improved regeneration of eggplant doubled haploids from microspore-derived calli [through organogenesis. Plant Cell Tissue Organ Cult. 122 (3), 759–765](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0320).

Rivas-Sendra, A., Campos-Vega, M., Calabuig-Serna, A., Segui-Simarro, J.M., 2017. Development and characterization of an eggplant (*Solanum melongena*) doubled

*Scientia Horticulturae 272 (2020) 109472*

[haploid population and a doubled haploid line with high androgenic response.](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0325)

[Euphytica 213 (4), 89](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0325).

Roberts-Oehlschlager, S.L., Dunwell, J.M., Faulks, R., 1990. Changes in the sugar content of barley anthers during culture on different carbohydrates. Plant Cell Tissue Organ [Cult. 22 (2), 77–85](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0330).

Rotino, G.L., 1996. Haploidy in eggplant. In: Jain, S.M., Sopory, S.K., Veilleux, R.E. (Eds.), *In vitro* Haploid Production in Higher Plants. Kluwer Academic, Dordrecht, pp.[115–141](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0335).

Rotino, G.L., 2016. Anther culture in eggplant (*Solanum melongena* L.). In *In Vitro* [Embryogenesis in Higher Plants. Humana Press, New York, NY, pp. 453–466](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0340).

Rotino, G.L., Sihachakr, D., Rizza, F., Vale, G., Tacconi, M.G., Alberti, P., et al., 2005. Current status in production and utilization of dihaploids from somatic hybrids be-[tween eggplant (*Solanum melongena L.)* and its wild relatives. Acta Physiol. Plant. 27](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0345)

[(4), 723–733](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0345).

Saini, D.K., Kaushik, P., 2019. Visiting eggplant from a biotechnological perspective: a [review. Sci. Hortic. 253, 327–340](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0350).

Salas, P., Prohens, J., Segui-Simarro, J.M., 2011. Evaluation of androgenic competence through anther culture in common eggplant and related species. Euphytica 182, [261–274](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0355).

Salas, P., Rivas-Sendra, A., Prohens, J., Segui-Simarro, J.M., 2012. Influence of the stage for anther excision and heterostyly in embryogenesis induction from eggplant anther [cultures. Euphytica 184, 235–250](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0360).

Scott, P., Lyne, R.L., Ap Rees, T., 1995. Metabolism of maltose and sucrose by microspores [isolated from barley (*Hordeum vulgare* L.). Planta 197 (3), 435–441](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0365).

Segui-Simarro, J.M., 2016. Androgenesis in solanaceae. *In Vitro* Embryogenesis in Higher [Plants. Humana Press, New York, NY, pp. 209–244](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0370).

[Segui-Simarro, J.M., Corral-Martinez, P., Parra-Vega, V., Gonzalez-Garcia, B., 2011.](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0375)

[Androgenesis in recalcitrant solanaceous crops. Plant Cell Rep. 30, 765–778](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0375).

Supena, E.D.J., Suharsono, S., Jacobsen, E., Custers, J.B.M., 2006. Successful develop-ment of a shed-microspore culture protocol for doubled haploid production in [Indonesian hot pepper (*Capsicum annuum* L.). Plant Cell Rep. 25 (1), 1–10](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0380).

Takahashi, Y., Yokoi, S., Takahata, Y., 2012. Effects of genotypes and culture conditions on microspore embryogenesis and plant regeneration in several subspecies of *Brassica* [*rapa* L. Plant Biotechnol. Rep. 6 (4), 297–304](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0385).

Taskin, H., Buyukalaca, S., Keles, D., Ekbic, E., 2011. Induction of microspore-derived embryos by anther culture in selected pepper genotypes. Afr. J. Biotechnol. 10 (75), [17116–17121](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0390).

Tipirdamaz, R., Ellialtioglu, S., 1998. The Effects of Cold Treatments and Activated Charcoal on Aba Contents of Anthers and *in Vitro* Androgenesis in Eggplant (*Solanum* [*melongena* L.). In Progress in Botanical Research. Springer, Dordrecht, pp. 607–610](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0395).

Tuberosa, R., Sanguineti, M.C., Conti, S., 1987. Anther culture of eggplant (*Solanum* [*melongena* L.) lines and hybrids. Genet Agrar 41 (3), 267–274](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0400).

Vural, G.E., Ari, E., Zengin, S., Ellialtioglu, S.S., 2019. Development of Androgenesis Studies on Eggplant (*Solanum Melongena* L.) in Turkey From Past to Present. In Sustainable Crop Production. IntechOpen[https://doi.org/10.5772/intechopen.](https://doi.org/10.5772/intechopen.88299)

[88299](https://doi.org/10.5772/intechopen.88299).

Wang, Y., Tong, Y., Li, Y., Zhang, Y., Zhang, J., Feng, J., Feng, H., 2011. High frequency plant regeneration from microspore-derived embryos of ornamental kale (*Brassica* [*oleracea*L. var. acephala). Sci. Hortic. 130 (1), 296–302](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0410).

Wu, L.M., Wei, Y.M., Zheng, Y.L., 2006. Effects of silver nitrate on the tissue culture of [immature wheat embryos. Russ. J. Plant Physiol. 53 (4), 530–534](http://refhub.elsevier.com/S0304-4238(20)30300-9/sbref0415).

Zhang, P., Fu, A.G., Wang, A.G., 1997. Role and possible mechanism of AgNO3 in plantculture *in vitro*. Plant Physiol Commun 33, 376–379.

14