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Callogenesis optimization of some globe artichoke [*Cynara cardunculus* var. *scolymus* (L.) Fiori] cultivars based on *in vivo* and *in vitro* leaf explants

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Abstract

Globe artichoke's [*Cynara cardunculus* var. *scolymus* (L.) Fiori] leaves are rich in polyphenols and due to health-promoting properties artichoke growing has been gaining interest. Optimization and development of valuable bioactive components, which are not in the standard amount in raw material can be achieved and increased with the assistance of *in vitro* techniques such as callus and subsequently cell suspension cultures. Therefore, in the present study *in vitro* callogenesis optimization of three globe artichoke cultivars was studied by using 29 different media combinations, based on basic Gamborg B5 medium supplemented with various concentrations of 1-Naphthaleneacetic acid (NAA), 6-Benzylaminopurine (BAP), 2,4-Dichlorophenoxyacetic acid (2,4-D), and Kinetin. Comparisons were made on the basis of using *in vivo* and *in vitro* leaves as explant material. In the experiment several parameters such as leaf explants development (%), callus formation (%), and callus weight (g) were assessed for each related cultivar. Results revealed that having auxin: cytokinin concentrations together at enough and well-balanced, having equal amounts or 10:1 concentrations of auxin: cytokinin, concentrations in media combinations are indispensable for stimulating the callogenesis in globe artichoke. The findings of the present study clearly revealed that, there were differences among cultivars regarding callus induction by using *in vivo* and *in vitro* leaf explants while *in vivo* leaf explants came into prominence regarding callus formation and weights. It is assumed that the findings of the present study may play a complementary and auxiliary role in several areas such as pharmaceutical engineering of globe artichoke.

Keywords: callus culture; callus induction; *Cynara cardunculus* var. *scolymus* (L.) Fiori; globe artichoke; *in vitro*; *in vivo*; leaf explant

Introduction

It is a well-known fact that plants have been at the service of humankind for food, health, and many other purposes. In order to ensure the adequate and balanced nutrition of world population, it has been always an aim to increase the agricultural production, quality and yield of the products by using genetic and biotechnological methods. Biotechnological applications are treasured for not only agricultural aspects but also non-nutritional purposes. Several biotechnological applications, including also callus culture technique, contribute to improvement of plants' agricultural properties.

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Callus culture-based technologies offer a broad range of usage in many fields such as agriculture and pharmacology studies on industrial scale. For example, callogenesis mediates micropropagation and can enable rapid multiplication of important agricultural and horticultural cultivars. Callogenesis is also a valuable tool for generating "specialized metabolites" by virtue of containing enough homogenous and dedifferentiated cells. Additionally, callogenesis studies are thought to be beneficial for comprehending the metabolic pathways of specialized metabolite (Sa *et al.*, 2001; Meratan *et al.*, 2009; Tariq *et al.*, 2014). In this content callus cultures may be utilized to generate the sustainable, large-scale, and marketable products of specialized metabolites to meet many needs of people in many fields such as food, agriculture, pharmaceutical, cosmetics, therapeutic (Espinosa-Leal *et al.*, 2018; Efferth, 2019). The artichoke has some health-improving properties thanks to the high amount of specialized metabolites present in different parts of the plant such as heads and leaves, therefore serves as a remarkable natural antioxidant source (Pandino *et al.*, 2012).

Plant growth regulators (PGRs) play an important role in callogenesis like in other tissue culture techniques. It is reported that when callus culture media relatively consist of high auxin and low cytokinin doses, cultured explants are induced for callogenesis (Farhan *et al.*, 2018). Callus formation can be stimulated by virtue of the specific PGRs at appropriate concentrations, genotypes and explant types (Ananthi *et al.*, 2011; Tariq *et al.*, 2014).

For callogenesis auxins have been considered as essential PGRs. Among auxins, NAA and 2,4-D are generally used to promote callus formation. On the other hand, cytokinins play a crucial mission in stimulating the cell division and subsequently accelerate the process of development of calli. In this regards, BAP and kinetin come into prominence among cytokinins used in callus cultures (Lestari *et al.*, 2019). In previous studies, researchers assessed various auxin and cytokinin combinations in different media for callus formation in several plants. Regarding artichoke callus formation, Gamborg B5 medium with BA + 2,4-D (1:10) was found to be best (Figueiredo *et al.*, 1987; Cimino *et al.*, 2006). In another study, it was reported that 2,4-D was effective regarding both cell development and anthocyanin biosynthesis in callus cultures of *Hibiscus sabdariffa* plants (Mizukami *et al.*, 1988; Siatka, 2019). In another research, the combination of BA and NAA was more effective in phenolic accumulation than BA alone in callus cultures of *Malus sieversii* (Ji *et al.*, 2015; Siatka, 2019). Previous studies conducted on artichoke clearly revealed that species, genotype, doses and types of PGRs had an influence on callus formation (Ruta *et al.*, 2013; Joshaghani *et al.*, 2014; Farhan *et al.*, 2018).

In the literature studies on callus induction and formation of globe artichoke and other species were mostly conducted by using *in vitro* leaf explants, while the limited number of studies was carried out with *in vivo* leaves. To our knowledge, there are a few studies, if there is any, comparing effects of *in vivo* and *in vitro* leaf explants on callus induction and formation in globe artichoke. Thus, the present study aimed to optimize the callus induction while comparatively evaluating the response of *in vivo* and *in vitro* leaf explants to callogenesis process in globe artichoke.

Materials and Methods

Plant material and surface sterilization

Three different globe artichoke cultivars, namely open-pollinated (OP) cultivars 'Bayrampaşa' and 'Sakız' and F_1 hybrid cultivar 'Olympus', were used as plant materials. *In vitro* and *in vivo* leaves were used as explant materials for callus induction and formation. To serve the purpose, special attention had been paid to collect the leaves that were newly formed and located in the innermost part of the plants. *In vivo* leaf explants separately collected for each cultivar were taken to the tissue culture laboratory and soil originating dirt was removed by keeping them under running tap water for 15 minutes. Afterwards *in vivo* leaf explants were kept in a solution of antibacterial soap (95 mL water + 5 mL antibacterial soap) for 15 minutes. After rinsing they were removed to laminar flow cabinets for surface sterilization which was conducted by using 20% sodium hypochlorite solution (5% active substance) for 10 minutes, followed by 3 rinsing with sterile distilled water.

In order to obtain *in vitro* leaf explants artichoke seeds were cultured in Murashige and Skoog (1962) medium (MS) with no PGRs. Before culturing the seeds were subjected to surface sterilization in a laminar flow cabinet by applying 20% sodium hypochlorite solution (5% active substance) for 20 minutes and rinsed with sterile distilled water 3 times. After seed germination and true leaf formation, leaves were removed and used as explant.

Preparing the media, establishment of callus cultures and culture conditions

In the present study, 29 different media (including control) combinations were used to optimize the callus formation in globe artichoke (Table 1). While Gamborg B5 (Gamborg *et al.*, 1968) was used as the basic media supplemented with different concentrations of BAP, kinetin, 2,4-D, and NAA. Leaf explants at the size of 0.5-1.0 cm, as used in previous studies (Abbas *et al.*, 2018; Sarmadi *et al.*, 2018), were placed in prepared culture medium and taken to growing room conditions with 24 ± 2 °C temperature, 16 hours light and 8 hours dark photoperiod under 3000 μ E.m^{-2}.s^{-1} light intensity.

Table 1. Media combinations used for callogenesis

Media Codes	Media - Gamborg B5 (g L^{-1})	Media composition					
		BAP (mg L^{-1})	2,4-D (mg L^{-1})	NAA (mg L^{-1})	Kin (mg L^{-1})	Glutamine (g L^{-1})	Sucrose (g L^{-1})
1 (Control)	3.2	-	-	-	-	-	30.0
2	3.2	0.1	1.0	-	-	-	30.0
3	3.2	0.5	5.0	-	-	-	30.0
4	3.2	1.0	1.0	-	-	-	30.0
5	3.2	2.0	2.0	-	-	-	30.0
6	3.2	5.0	5.0	-	-	-	30.0
7	3.2	0.1	1.0	-	-	1.0	30.0
8	3.2	-	6.0	-	0.5	-	30.0
9	3.2	1.0	-	7.0	-	-	30.0
10	3.2	0.5	-	5.0	-	-	30.0
11	3.2	5.0	-	5.0	-	-	30.0
12	3.2	0.1	-	0.5	-	-	30.0
13	3.2	0.1	-	1.0	-	-	30.0
14	3.2	0.1	-	2.0	-	-	30.0
15	3.2	0.5	-	0.5	-	-	30.0
16	3.2	0.5	-	1.0	-	-	30.0
17	3.2	0.5	-	2.0	-	-	30.0
18	3.2	1.0	-	1.0	-	-	30.0
19	3.2	1.0	-	2.0	-	-	30.0
20	3.2	2.0	-	2.0	-	-	30.0
21	3.2	-	0.5	-	0.1	-	30.0
22	3.2	-	1.0	-	0.1	-	30.0
23	3.2	-	2.0	-	0.1	-	30.0
24	3.2	-	0.5	-	0.5	-	30.0
25	3.2	-	1.0	-	0.5	-	30.0
26	3.2	-	2.0	-	0.5	-	30.0
27	3.2	-	1.0	-	1.0	-	30.0
28	3.2	-	2.0	-	1.0	-	30.0
29	3.2	-	2.0	-	2.0	-	30.0

*Abbreviations: BAP: 6-Benzylaminopurine, 2,4-D: 2,4-Dichlorophenoxyacetic acid, NAA: 1-Naphthaleneacetic acid, Kin: Kinetin

Evaluated parameters

Leaf explants and callus development (%), fresh weights (g) of formed callus were assessed with nearly 30-day intervals after each subculture. A total of three subcultures were conducted to increase the amount of calli obtained. The following equations were used for determining the leaf explants development (1) and callus induction (2) percentages:

(1) Leaf explant development (%) = (number of developed leaf explants/ number of total cultured leaf explants) × 100

(2) Callus induction (%) = (number of induced callus explants/ number of developed callus explants) × 100

Statistical analysis

The experiment was performed as three replicates. Three petri dishes with 20 leaf explants were used in each replicate. A total of three subcultures were conducted by using processes for both *in vivo* and *in vitro* leaf explants. At the end of three subcultures average values were calculated and obtained data were subjected to variance analysis in the JMP package program and the differences between the averages were determined by LSD test.

Results

As stated earlier the callus culture method is an *in vitro* culture technique that has high potential in several areas particularly pharmaceutical studies of different crops, including artichoke. In the previous studies, it was hypothesized that artichoke's leaves had high potential regarding valuable bioactive metabolites which could be used in pharmaceutics. At the beginning of the research, when we were setting up the experiments we speculated that explants originating from *in vitro* culture may have more tendencies to callogenesis than the *in vivo* leaf explants. And that is why the results and discussion were based on in the comparisons of *in vitro* and *in vivo* leaf explants as well as setting up an optimized callogenesis protocol.

Effect of disinfection protocol on explants

In present study there was no surface sterilization procedure for *in vitro* leaf explants since they originated from *in vitro* conditions and were free of any contaminants. On the other hand, special attention was given to surface sterilization of *in vivo* leaf explants. To serve the purpose first of all soil-based contamination sources were removed by keeping explants under running tap water. And then applying stated disinfection protocol resulted with healthy explants and that is why no serious risk of disinfection took place throughout the experiments.

Evaluations of in vitro and in vivo leaf explants development

Regarding explants types, responds of *in vivo* leaf explants were better than *in vitro* leaf explants in a comparison based on explants development among all cultivars and media combinations were tested (Table 2).

Experimental results also clearly revealed that there were statistically significant differences among cultivars in terms of explants development regardless of being *in vivo* or *in vitro* within all media combinations tested. The well responded cultivar was 'Bayrampaşa' OP, followed by 'Sakız' OP and 'Olympus' F_1, respectively.

If one considers effects of 29 media combinations, Gamborg B5 medium supplemented with 0.5 mg L^{-1} BAP + 5.0 mg L^{-1} NAA (medium No.10) was found to be superior to other media combinations in both explant types and for all cultivars.

Table 2. Leaf explants development based on media combinations and cultivars (%)

Cultivars		'Bayrampaşa' OP		'Sakız' OP		'Olympus' F_1		Mean values of media
Leaf explants		*in vivo*	*in vitro*	*in vivo*	*in vitro*	*in vivo*	*in vitro*	
Media Numbers	1	22.04 l-x	22.63 l-u	22.22 l-w	0 l-V	6.65 l-R	0 l-V	12.26 *B*
	2	38.47 T	71.62 a	46.25 y	0 l-V	57.86 n	0 l-V	35.70 *E*
	3	60.05 g	45.23 A	22.77 l-t	0 l-V	34.99 Y	0 l-V	27.17 *O*
	4	47.55 w	60.87 f	57.31 o	0 l-V	55.27 p	0 l-V	36.83 *D*
	5	39.53 O	59.61 i	59.90 h	11.19 l-M	58.25 m	0 l-V	38.08 *B*
	6	33.51 b	40.47 M	61.14 e	0 l-V	40.63 L	11.10 l-M	31.14 *J*
	7	25.54 l-p	58.25 m	57.21 o	0 l-V	48.97 u	0 l-V	31.66 *I*
	8	65.61 d	51.91 s	39.02 Q	0 l-V	22.21 l-w	0 l-V	29.79 *K*
	9	47.43 w	45.00 B	69.34 c	0 l-V	35.65 W	0 l-V	32.90 *F*
	10	43.43 F	53.74 q	58.85 k	14.45 l-G	47.84 v	11.14 l-M	38.24 *A*
	11	44.45 C	22.44 l-v	69.54 b	15.55 l-F	59.34 j	16.64 l-E	37.99 *C*
	12	27.21 lm	32.99 d	38.27 U	4.48 l-U	8.88 l-O	8.96 l-O	20.13 *Y*
	13	4.99 l-T	41.95 H	38.60 S	4.41 l-U	8.88 l-O	8.93 l-O	17.96 *A*
	14	18.13 l-B	31.69 f	30.91 g	5.57 l-S	11.29 l-L	12.24 l-K	18.30 *Z*
	15	18.28 l-A	35.68 W	43.81 E	13.36 l-I	13.55 l-H	19.76 l-z	24.07 *T*
	16	6.94 l-Q	47.24 x	33.44 bc	6.68 l-R	30.55 h	13.56 l-H	23.07 *V*
	17	5.54 l-S	39.28 P	25.53 l-p	17.74 l-C	12.77 l-J	8.96 l-O	18.30 *Z*
	18	39.32 P	29.89 j	58.68 l	6.65 l-R	44.24 D	13.35 l-I	32.02 *H*
	19	24.10 l-s	29.19 l	32.94 d	6.67 l-R	52.95 r	17.79 l-C	27.27 *N*
	20	17.29 l-D	40.47 M	26.29 l-o	8.88 l-O	41.65 I	13.35 l-I	24.65 *S*
	21	45.18 A	40.70 L	29.39 k	6.67 l-R	22.21 l-w	13.37 l-I	26.25 *Q*
	22	33.32 c	30.15 i	39.75 N	8.87 l-O	40.88 K	15.53 l-F	28.08 *M*
	23	32.77 e	30.22 i	24.55 l-q	6.67 l-R	43.87 E	13.38 l-I	25.24 *R*
	24	20.10 l-y	12.36 l-K	41.29 J	5.54 l-S	39.04 Q	11.09 l-M	21.57 *X*
	25	24.43 l-r	41.66 I	48.95 u	6.67 l-R	26.54 l-n	13.34 l-I	26.93 *P*
	26	38.88 R	18.14 l-B	29.23 l	8.92 l-O	34.62 Z	13.35 l-I	23.86 *U*
	27	42.98 G	34.41 a	49.82 t	7.64 l-P	45.66 z	15.56 l-F	32.68 *G*
	28	35.24 X	38.26 U	27.25 lm	8.93 l-O	10.66 l-N	15.52 l-F	22.64 *W*
	29	24.49 l-r	37.24 V	44.31 D	11.13 l-M	38.50 ST	15.56 l-F	28.54 *L*
Mean values	Cultivars	'Bayrampaşa' OP = 35.69 A 'Sakız' OP = 24.36 B 'Olympus' F_1 = 22.01 C						
	Leaf exp.	*in vivo* = 36.18 A *in vitro* = 18.53 B						
LSD values		$LSD_{cultivar} = 0.0157$ $LSD_{explant} = 0.0128$ $LSD_{media} = 0.0488$						

Different letters among cultivars, media compositions and leaf explants denote significant differences (LSD test, $p < 0.05$).

Callus formation

Experimental results originating from callus culture revealed that the role of PGRs added to the nutrient medium greatly influenced the initial callus induction and formation. There were statistically significant differences in callus formation percentages on the basis of cultivars, leaf explants and among media combinations (Table 3). Regarding cultivars, the best callus formation percentage was obtained from 'Bayrampaşa' OP cultivar, while the medium supplemented with 0.5 mg L^{-1} BAP + 5.0 mg L^{-1} NAA (medium No.10) was at the forefront among media combinations. The important point of the stated medium was to have 10:1 concentrations of auxin: cytokinin.

No callus formation was obtained by using *in vitro* leaf explants of 'Olympus' F_1. A similar trend was found for *in vitro* leaf explants of 'Sakız' OP cultivar with exceptions medium No.15 (0.5 mg L^{-1} BAP + 0.5 mg L^{-1} NAA) and medium No.17 (0.5 mg L^{-1} BAP + 2.0 mg L^{-1} NAA).

Table 3. Callus formation of leaf explants based on media combinations and cultivars (%)

Cultivars		'Bayrampaşa' OP		'Sakız' OP		'Olympus' F_1		Mean values of media
Leaf explants		*in vivo*	*in vitro*	*in vivo*	*in vitro*	*in vivo*	*in vitro*	
Media Numbers	1	0 l-D	0 l-D	3.33 l-B	0 l-D	0 l-D	0 l-D	0.55 *C*
	2	26.49 i	33.22 Y	54.04 o	0 l-D	59.39 j	0 l-D	28.85 *I*
	3	39.63 N	34.26 S	33.89 T	0 l-D	25.55 j	0 l-D	22.22 *P*
	4	49.76 t	37.30 Q	79.02 b	0 l-D	52.31 q	0 l-D	36.39 *B*
	5	33.45 V-X	46.58 z	70.27 c	0 l-D	55.82 l	0 l-D	34.35 *C*
	6	25.21 k	0 l-D	62.87 g	0 l-D	33.67 U	0 l-D	20.29 *S*
	7	33.33 XY	49.73 t	59.11 k	0 l-D	40.26 L	0 l-D	30.40 *G*
	8	47.41 x	61.96 i	21.90 l-n	0 l-D	16.65 l-u	0 l-D	24.65 *M*
	9	43.25 F	47.66 w	66.15 e	0 l-D	30.58 d	0 l-D	31.27 *E*
	10	42.32 I	62.31 h	65.00 f	0 l-D	54.18 o	0 l-D	37.30 *A*
	11	49.83 st	0 l-D	86.98 a	0 l-D	48.56 v	0 l-D	30.89 *F*
	12	33.32 XY	31.97 b	33.63 U	0 l-D	0 l-D	0 l-D	16.48 *Y*
	13	0 l-D	43.29 F	20.21 l-p	0 l-D	0 l-D	0 l-D	10.58 *B*
	14	20.82 l-o	43.32 F	32.85 Z	0 l-D	11.10 l-x	0 l-D	18.01 *V*
	15	18.05 l-t	42.60 H	40.72 K	0.90 l-C	0 l-D	0 l-D	17.04 *X*
	16	8.01 l-z	42.10 J	19.79 l-q	0 l-D	7.38 l-A	0 l-D	12.88 *A*
	17	10.17 l-y	43.67 E	33.33 XY	3.47 l-B	0 l-D	0 l-D	15.10 *Z*
	18	53.23 p	30.94 c	49.94 s	0 l-D	45.54 B	0 l-D	29.94 *H*
	19	43.41 F	33.41 WX	30.97 c	0 l-D	47.21 y	0 l-D	25.83 *K*
	20	19.04 l-r	32.55 a	27.29 h	0 l-D	31.93 b	0 l-D	18.47 *U*
	21	55.53 m	29.48 ef	24.61 l	0 l-D	18.49 l-s	0 l-D	21.35 *R*
	22	30.54 d	32.49 a	49.06 u	0 l-D	33.33 XY	0 l-D	24.23 *N*
	23	29.62 e	33.62 UV	40.85 K	0 l-D	31.09 c	0 l-D	22.53 *O*
	24	22.21 lm	33.57 U-W	46.24 A	0 l-D	29.00 g	0 l-D	21.83 *Q*
	25	14.80 l-w	35.69 R	51.83 r	0 l-D	16.65 l-u	0 l-D	19.83 *T*
	26	15.54 l-v	38.92 O	18.32 l-s	0 l-D	33.32 XY	0 l-D	17.68 *W*
	27	55.07 n	44.04 D	49.09 u	0 l-D	44.42 C	0 l-D	32.10 *D*
	28	43.00 G	66.43 d	29.38 f	0 l-D	11.10 l-x	0 l-D	24.98 *L*
	29	53.07 p	32.95 Z	40.00 M	0 l-D	37.75 P	0 l-D	27.29 *J*
Mean values	Cultivars	'Bayrampaşa' OP = 34.14 A		'Sakız' OP = 21.46 B		'Olympus' F_1 = 14.05 C		
	Leaf exp.	*in vivo* = 34.16 A			*in vitro* = 12.28 B			
LSD values		$LSD_{cultivar} = 0.0231$		$LSD_{explant} = 0.0188$		$LSD_{media} = 0.0718$		

Different letters among cultivars, media compositions and leaf explants denote significant differences (LSD test, $p < 0.05$).

Evaluation of callus weights

When the resulting calli were evaluated in terms of their weight, there were statistically significant differences among the cultivars, media combinations and between explant types (Table 4). The medium No.11, enriched with 5.0 mg L^{-1} BAP + 5.0 mg L^{-1} NAA, was the best among all 29 media combinations regarding callus weights, while *in vivo* leaf explants had the better result than *in vitro* leaf explants (Figure 1). The heaviest callus weight was obtained from *in vivo* leaf explants of 'Bayrampaşa' OP cultivar.

No desired results were obtained with all tested media combinations for 'Sakız' OP and 'Olympus' F_1 cultivars for *in vitro* leaf explants regarding callus formation and weights.

Table 4. Callus weights of leaf explants based on media combinations and cultivars (g)

Cultivars		'Bayrampaşa' OP		'Sakız' OP		'Olympus' F_1		Mean values of media
Leaf explants		*in vivo*	*in vitro*	*in vivo*	*in vitro*	*in vivo*	*in vitro*	
Media Numbers	1	0 V	0 V	0.008 UV	0 V	0 V	0 V	0.0014 *Q*
	2	0.051 y-F	0.183 gh	0.066 vwx	0 V	0.060 w-B	0 V	0.060 *G*
	3	0.060 w-B	0.106 nop	0.015 R-U	0 V	0.024 O-S	0 V	0.034 *MN*
	4	0.077 s-v	0.306 c	0.118 lm	0 V	0.045 D-J	0 V	0.091 *D*
	5	0.040 F-L	0.176 h	0.100 pq	0 V	0.035 J-O	0 V	0.058 *GH*
	6	0.070 t-w	0 V	0.081 rst	0 V	0.061 w-A	0 V	0.035 *MN*
	7	0.340 b	0.156 i	0.200 f	0 V	0.040 F-L	0 V	0.122 *B*
	8	0.156 i	0.191 fg	0.036 I-N	0 V	0.030 L-P	0 V	0.069 *F*
	9	0.248 d	0.114 mno	0.230 e	0 V	0.087 rs	0 V	0.113 *C*
	10	0.076 s-v	0.192 fg	0.127 jkl	0 V	0.098 pq	0 V	0.082 *E*
	11	0.573 a	0 V	0.336 b	0 V	0.054 y-E	0 V	0.160 *A*
	12	0.048 C-H	0.028 M-Q	0.040 F-L	0 V	0 V	0 V	0.019 *P*
	13	0 V	0.068 uvw	0.030 L-P	0 V	0 V	0 V	0.016 *P*
	14	0.036 I-N	0.048 C-H	0.040 F-L	0 V	0.034 J-O	0 V	0.026 *O*
	15	0.024 O-S	0.061 w-A	0.078 stu	0.065 wxy	0 V	0 V	0.038 *LM*
	16	0.015 R-U	0.064 wxy	0.018 Q-U	0 V	0.026 N-R	0 V	0.020 *P*
	17	0.013 STU	0.063 wxy	0.034 J-O	0.013 TU	0 V	0 V	0.020 *P*
	18	0.090 qr	0.045 D-J	0.130 jk	0 V	0.064 wxy	0 V	0.054 *HI*
	19	0.067 u-x	0.028 M-Q	0.044 D-J	0 V	0.064 wxy	0 V	0.034 *MN*
	20	0.035 J-O	0.037 H-M	0.045 D-J	0 V	0.043 E-J	0 V	0.026 *O*
	21	0.117 lmn	0.040 F-L	0.040 F-L	0 V	0.050 A-F	0 V	0.041 *KL*
	22	0.055 y-D	0.043 F-K	0.120 klm	0 V	0.054 y-E	0 V	0.045 *JK*
	23	0.048 C-H	0.077 s-v	0.044 D-J	0 V	0.028 M-Q	0 V	0.033 *N*
	24	0.047 C-I	0.055 y-D	0.130 jk	0 V	0.040 F-L	0 V	0.052 *I*
	25	0.032 K-O	0.118 lm	0.090 qr	0 V	0.038 G-M	0 V	0.046 *J*
	26	0.077 s-v	0.062 w-z	0.020 P-T	0 V	0.040 F-L	0 V	0.033 *N*
	27	0.080 rst	0.049 B-G	0.135 j	0 V	0.102 p	0 V	0.061 *G*
	28	0.056 x-C	0.106 op	0.040 F-L	0 V	0.010 TUV	0 V	0.035 *MN*
	29	0.050 A-F	0.041 F-L	0.054 y-E	0 V	0.064 wxy	0 V	0.034 *MN*
Mean values	Cultivars	'Bayrampaşa' OP = 0.087 A 'Sakız' OP = 0.043 B 'Olympus' F_1 = 0.021 C						
	Leaf exp.	*in vivo* = 0.071 A *in vitro* = 0.029 B						
LSD values		$LSD_{cultivar}$ = 0.001455 $LSD_{explant}$ = 0.001188 LSD_{media} = 0.004526						

Different letters between cultivars, media compositions and leaf explants denote significant differences (LSD test, $p < 0.05$).

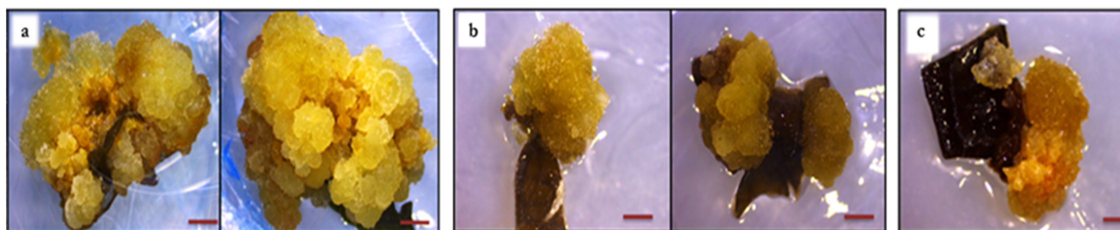

Figure 1. Callus formation of *in vivo* leaf explants on medium No.10; **a.** 'Bayrampaşa' OP, **b.** 'Sakız' OP; **c.** 'Olympus' F_1

Discussion

It is known that many factors are on the induction and regeneration of calli as well as secondary metabolite formation and accumulation in explants cultured *in vitro*. Among these factors, genotype, explant type, media composition and combination, carbohydrate source, type and concentration of PGRs, and culture conditions were reported to play an important role (Rueb *et al.*, 1994; Shahsavari *et al.*, 2010; Siatka, 2019).

In many studies conducted on obtaining callus on different plants, it was reported that callus could not be obtained in nutrient media without PGRs (Fiegert *et al.*, 2000; Yasmin *et al.*, 2003; Elaleem *et al.*, 2009). It has also reported that cultured media should be supplemented with PGRs at the appropriate dose and combination to promote callus induction and plant regeneration, depending on genotype and explant type (Lestari *et al.*, 2019). A number of studies have been carried out by researchers emphasizing the importance of auxins and cytokinins to get callus formation, development, and maintenance (Joshaghani *et al.*, 2014; Baharan *et al.*, 2015; Efferth, 2019). In the present study we also tested several PGRs at different types and concentrations and findings clearly revealed that the use of PGRs is a must for callogenesis optimization in artichoke. Findings of present study, therefore, were in agreement with previous studies (Ruta *et al.*, 2013; Joshaghani *et al.*, 2014; Abbas *et al.*, 2018). It is clearly seen that PGRs play a critical role in the callogenesis process. Previous studies reported that using auxins and cytokinins together in various proportions promote callus induction, especially using varying amounts of NAA and BAP provide effective callus formation in different plants. Hesami and Daneshvar (2018) reported that they obtained maximum callus formation in the combination of 1.5 mg L^{-1} NAA + 0.15 mg L^{-1} BAP in their study. Wani *et al.* (2018) stated that the use of *in vivo* leaf explants provided the best results (83.5% callus formation rate) in the 1:1 rate of NAA + BAP combination.

Findings of the current study showed that three artichoke cultivars, regardless of explant types well responded to callogenesis in terms of callus induction in media No.10 (0.5 mg L^{-1} BAP + 5.0 mg L^{-1} NAA). Regarding PGRs, providing a balance between auxin and cytokinin is a necessity to achieve a successful callus formation for all three cultivars used in present study. NAA + BAP combination at 10:1 rate triggered callus induction and subsequently formation. On the other hand, NAA + BAP combination at 1:1 rate was found to be highly effective on increasing callus weights for all cultivars.

According to these findings, appropriate PGRs combinations can be alternatively used based on the purpose of study. For example, if someone wants to achieve callus induction and formation can use NAA + BAP combination at 10:1 rate. But if someone needs callus at large quantities, for example to be used in cell suspension cultures, should use NAA + BAP combination at the rate of 1:1.

As stated earlier one of the main aims of the present study was to evaluate the effects of *in vitro* and *in vivo* leaf explants to callogenesis in artichoke. Obtained results indicated that responses of *in vivo* leaf explants to callogenesis were better than *in vitro* leaf explants, meaning *in vivo* leaf explants may be preferable for further studies.

Among all 29 media combinations tested in present study medium No.7 was the only medium having glutamine. In previous plant tissue culture studies when plant cells needed, glutamine plays an alternative energy supporter role while supplying protein and nucleic acid synthesis at great number, that trigger and maintain the plant cell functions for augmenting the formation of the embryo, embryonic calli, organs, and plantlets in many plant species (Ogita *et al.*, 2001; Newsholme *et al.*, 2003; Vasudevan *et al.*, 2004; Hamasaki *et al.*, 2005; Mazri *et al.*, 2016; Borpuzari *et al.*, 2018). Glutamine was also successfully used as an alternative nitrogen source at various concentrations for improving *in vitro* carrot cell-suspension cultures (Olsen, 1987; Ozsan and Onus, 2018) and that is why we wanted to see the effects of glutamine on callus formation which is a must for cell suspension cultures. When the effects of glutamine on callus induction for all three cultivars and both explant types were examined, glutamine did not have any superior effect. On the other hand, as can be seen in Table 4 glutamine had positive effect on callus weight of ‘Bayrampaşa’ OP with *in vivo* leaf explant.

Results may reflect that different concentrations of glutamine should be tested on various artichoke cultivars for further studies in order to clearly reveal the effects of glutamine.

For the majority of plant tissue culture studies two media have fore fronted: Murashige & Skoog (MS) and Gamborg B5. In previous studies conducted by us using MS medium supplemented with same PGRs as indicated in material and method section failed as no callus induction and formation was obtained for three cultivars used in present study (data not shown). That is why Gamborg B5 medium was preferred in present study. There may be different reasons for Gamborg B5 medium being successful. For example, Gamborg B5 medium's inorganic nutrients concentration is less than in MS medium. In addition, these two basic media differ from each other with respect to vitamins. Gamborg B5 medium has higher concentration of thiamine than the MS medium. Thiamine takes part in cell biosynthesis and metabolism and also maintains *in vitro* growth (Willims, 1995; Jacob and Malpathak, 2005).

Such differences can act as trigger for callus formation from the leaf explants, which is important in terms of bioactive compounds present in leaf.

Conclusions

The findings of the present study clearly revealed that there were differences among cultivars regarding callus induction by using *in vivo* and *in vitro* leaf explants while *in vivo* leaf explants came into prominence regarding callus formation and weights. Among media combinations, two of them have drawn attention; 0.5 mg L^{-1} BAP + 5.0 mg L^{-1} NAA (medium No.10) and 5.0 mg L^{-1} BAP + 5.0 mg L^{-1} NAA (medium No.11). But none of the media provided any callus formation for Olympus F_1 when *in vitro* leaf explants were used; meaning different media combinations should be optimized in accordance with the cultivar used for further studies. It is assumed that the findings of the present study may guide the people working on artichoke breeding/genetics by using callogenesis and those working on pharmaceutical engineering of globe artichoke by using cell suspension cultures.

Authors' Contributions

Both authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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