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Effects of Callus Cell Suspension Cultures and Elicitor Applications on Bioactive Components in Globe Artichoke [*Cynara cardunculus* var. *scolymus* (L.) Fiori]

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ABSTRACT

Globe artichoke [*Cynara cardunculus* var. *scolymus* (L.) Fiori] has many health-protecting properties due to its valuable bioactive components which are not stable and produced in high amounts in the raw plant material in nature. These bioactive components that gaining interest can be increased thanks to the contribution of valuable *in vitro* techniques, such as callus and cell suspension cultures, and various new applications such as elicitor treatments. The present study aimed to determined bioactive components in three globe artichoke cultivars by using callus cell suspension cultures in various media combinations and by applying two elicitor treatments, namely methyl jasmonate and chitosan, at 3 different concentrations (methyl jasmonate 50 µM, 100 µM, and 200 µM; chitosan 200 mg L^{-1}, 400 mg L^{-1}, and 800 mg L^{-1}) with 3 different application durations (24h, 48h, and 72h). The bioactive compounds profile of cultivars was determined by HPLC-DAD. Obtained results revealed that using well-balanced concentrations of auxin: cytokinin (1:1 or 10:1) in a media composition is a must for triggering the callus formation process for globe artichoke. Results also showed that accumulated bioactive components and their amounts varied based on cultivars. Experiment results revealed that different types of elicitors other than methyl jasmonate and chitosan, or different doses of elicitors and application durations should be used/tested to get desired levels of bioactive components. The findings of the present study may play a supportively and complementarily mission in several important fields such as agriculture, and pharmaceutical engineering.

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Kallus Hücre Süspansiyon Kültürleri ve Elisitör Uygulamalarının Enginarda [*Cynara cardunculus* var. *scolymus* (L.) Fiori] Biyoaktif Bileşenler Üzerine Etkisi

ÖZET

Enginar [*Cynara cardunculus* var. *scolymus* (L.) Fiori] doğada ham bitki materyalinde yüksek miktarlarda ve stabil olarak üretilmeyen değerli biyoaktif bileşenleri nedeniyle sağlık üzerine koruyucu birçok özelliğe sahiptir. Bu biyoaktif bileşenler, kallus ve hücre süspansiyon kültürleri gibi değerli *in vitro* tekniklerin ve elisitör gibi çeşitli yeni uygulamaların katkısıyla artırılabilmektedir. Bu çalışmada, çeşitli besi ortam kombinasyonlarında kallus hücre süspansiyon kültürleri kullanılarak ve 3 farklı konsantrasyonda (metil jasmonat 50 µM, 100 µM ve 200 µM; kitosan 200 mg L^{-1}, 400 mg L^{-1} ve 800 mg L^{-1}) 3 farklı uygulama süresiyle (24 saat, 48 saat ve 72 saat) uygulamaların biyoaktif içerikler üzerine olan etkisi araştırılmıştır. Çeşitlerin biyoaktif bileşik profili HPLC-DAD ile belirlenmiştir. Elde edilen sonuçlar, besi ortamı bileşiminde iyi dengelenmiş oksin: sitokinin (1:1 veya 10:1) konsantrasyonlarının kullanılmasının, enginarda kallus oluşum sürecini tetiklemek için bir zorunluluk olduğunu açıkça ortaya koymuştur. Sonuçlar ayrıca biriken biyoaktif bileşenlerin ve miktarlarının çeşitlere göre değiştiğini ortaya koymuştur. Araştırma sonuçları, istenen biyoaktif bileşen seviyelerini elde etmek için metil

Bahçe Bitkileri

Araştırma Makalesi

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Anahtar Kelimeler
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Flavonoidler
Kitosan
Metil jasmonat

jasmonat ve kitosan dışında farklı tipte elisitörlerin veya aynı elisitörlerin farklı dozlarının ve/veya uygulama sürelerinin kullanılması/test edilmesi gerektiğini açık bir şekilde ortaya koymuştur. Mevcut çalışmanın bulgularının, tarım ve ilaç mühendisliği gibi birçok önemli alanda destekleyici ve tamamlayıcı bir etkisi olacağı düşünülmektedir.

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INTRODUCTION

The globe artichoke [*Cynara cardunculus* var. *scolymus* (L.) Fiori] with special therapeutic features for human is native to Mediterranean Basin. Globe artichoke has gained its reputation because it has valuable bioactive components in its edible parts as well as its non-edible parts. Although its non-edible parts are treated as waste, their value has recently been more understood and has become important (Pandino et al., 2011; Pandino et al., 2013).

Plants incorporate precious bioactive compounds with healing properties on human are produced and used in many fields, incuding pharmaceuticals, agrochemicals, food flavor, texture, and color. But it is difficult to attain the desired level of bioactive compounds in plants and since it is dependent on the variation of environmental conditions, stresses, genotypes, and plant parts. To overcome such uncontrollable circumstances, biotechnological practices like callus and cell suspension culture techniques are used (Pandino et al., 2017; El-Bahr et al., 2018). However, plant parts from which explants are taken to initiate callus and cell suspension cultures, which also allow the production of bioactive compounds, are also important. Starting *in vitro* callus and cell suspension cultures using plant parts where the target bioactive compound is concentrated will be advantageous for the potential bioactive compound obtained during the culture (Sökmen and Gürel, 2001). In addition to using these morphologically unorganized and undifferentiated biotechnological techniques, other treatments namely precursors, elicitors, immobilizations, can also be used for cell growth and production of targeted bioactive compounds in large volumes. Considering the above stated potential of *in vitro* techniques, they serve as reliable, sustainable, continuous and standardized production processes of precious bioactive compounds.

The use of elicitors provides an opportunity for effective and practical work called elicitation. Using various types of elicitors (biotic and abiotic) at different doses and/or applying durations, changes, or enhancements in the amount of the desired bioactive component can be achieved (Naik and Al–Khayri, 2015). Two different elicitors were preferred in this study; methyl jasmonate and chitosan. Methyl jasmonate is a phytohormone which is a volatile methyl ester form of jasmonic acid, has many roles in the plant, and is known to play a critical role in a number of important physiological and developmental processes that take place in the plant. Chitin is a component formed in the wall structure of various plant fungal pathogens. Chitosan, on the other hand, is a component derived from chitin and is often used as an elicitor (Ahmed and Baig, 2014; Alsoufi et al., 2019).

Using these techniques has been practiced in many medicinal and aromatic plants from past to present and their use is getting increased day by day in basic fields such as agriculture, food, and pharmacology. Based on the literature search, it is found out that there are limited studies using callus cell suspension culture techniques and elicitor treatments in globe artichoke. The present study aimed to determine bioactive components in three globe artichoke cultivars by using callus cell suspension cultures in various media combinations and by applying two elicitor treatments.

MATERIAL and METHOD

Plant materials and surface sterilization

Two open-pollinated (OP) globe artichoke cultivars, namely 'Bayrampaşa' and 'Sakız', and one F_1 hybrid globe artichoke cultivar 'Olympus' were used as plant materials. For callus induction and formation, especially newly formed leaves were separately collected and carried to the tissue culture laboratory for surface sterilization. To serve the purpose: (1) leaf explants were kept under running tap water for 15 minutes, (2) in an antibacterial soap solution (5 mL antibacterial soap + 95 mL water) for 15 minutes. After rinsing, the leaf explants were taken to laminar airflow workbench for further surface sterilization process which was carried out by treating with 20% (v/v) of a commercial bleach solution (40 g/L active chlorine) for 10 minutes, followed by 3 times rinsing with sterilized distilled water (López-Pérez and Martínez JA 2015; Ozsan and Onus, 2020a; b).

Media compositions, induction and conditions of callus culture, biomass yield

For establishing the callus cultures from newly formed leaf explants, several media compositions were assessed according to Ozsan and Onus, (2020b). The medium compositions consisted of Gamborg B5 basic media (Gamborg et al., 1968) supplemented with plant growth regulators (BAP, NAA, Kin, 2,4-D) at various concentrations (0.1, 0.5, 1.0, 2.0 mg L^{-1}), and all medium combinations contained 30.0 g L^{-1} sucrose and 6 g L^{-1} plant agar, pH was adjusted to 5.8 before autoclaved. After the necessary surface sterilization processes, initial plant leaf explants were cut at the size of 0.5-1.0 cm (Abbas et al., 2018; Sarmadi et al., 2018) and placed on these medium combinations. Plant growth regulator-free Gamborg B5 basal medium (Gamborg B5-0) was used as a control medium. All callus cultures were incubated at a growth chamber having 24±2 °C temperature, 16 hours light and 8 hours dark photoperiod under 3000 µ $E.m^{-2}.s^{-1}$ light intensity.

Differences between callus morphology were recorded weekly intervals based on cultivars and media combinations. In about 3-4 weeks, among all media combinations, well-responded calli growths were recorded on various media combinations for each cultivar. Five sub-cultures were performed in the same media combinations for each cultivar. Formed calli were maintained and utilized for further steps of experiments.

For fresh weights biomass yield of calli belonging to cultivars, the harvested calli from each media composition were measured and it was recorded as fresh weights.

Establishment of cell suspension cultures

Calli developed from various media combinations as stated above were used for initiating the cell suspension cultures. Suspension cultures were established for each cultivar by transferring 1.0 g fresh weight of friable calli onto Gamborg B5 liquid medium; (50 mL) within 250 mL capacity of Erlenmeyer flasks. They were strengthened with several concentrations of plant growth regulators depending on each cultivar's response as down stated and based on findings of Ozsan and Onus, (2020b).

For 'Bayrampaşa' OP cultivar, (1) 1.0 mg L^{-1} BAP + 1.0 mg L^{-1} NAA (medium no 18), (2) 0.1 mg L^{-1} KIN + 1.0 mg L^{-1} 2,4-D (medium no 22), (3) 0.1 mg L^{-1} KIN + 2.0 mg L^{-1} 2,4-D (medium no 23), (4) 1.0 mg L^{-1} KIN + 1.0 mg L^{-1} 2,4-D (medium no 27), (5) 1.0 mg L^{-1} KIN + 2.0 mg L^{-1} 2,4-D (medium no 28).

For 'Sakız' OP cultivar, (1) 0.1 mg L^{-1} BAP + 1.0 mg L^{-1} NAA (medium no 13), (2) 1.0 mg L^{-1} BAP + 2.0 mg L^{-1} NAA (medium no 19), (3) 0.1 mg L^{-1} KIN + 1.0 mg L^{-1} 2,4-D (medium no 22), (4) 0.5 mg L^{-1} KIN + 1.0 mg L^{-1} 2,4-D (medium no 25), (5) 1.0 mg L^{-1} KIN + 1.0 mg L^{-1} 2,4-D (medium no 27), (6) 5.0 mg L^{-1} BAP + 5.0 mg L^{-1} NAA (medium no 6).

For 'Olympus' F_1 hybrid cultivar, (1) 1.0 mg L^{-1} BAP + 2.0 mg L^{-1} NAA (medium no 19).

These media combinations were chosen for present study because they were found to be highly effective on callus formation with good enough amounts (Ozsan and Onus, 2020b).

All Erlenmeyer flasks were kept on an orbital rotary shaker, at 130 rpm, in the growth chamber until full suspension. Afterwards suspended cells were filtered individually through 0.45 µm filters, washed out with sterile distilled water, bare down gently on sterilized filter paper to remove excess water, then weighed, and subsequently, cells were sub-cultured in a fresh medium. The stated process was repeated at the end of each sub-culture with 12-days intervals. For determining dry weights, cell suspension cultures were subjected to the oven-dry process at 60 °C until reaching the constant weights.

Preparation of elicitors' concentrations and elicitation

Stock solution of methyl jasmonate (MeJa, Sigma) was prepared by dissolving in 70% (v/v) ethanol. Distilled pure water was used for further dilutions. Filter sterilization with 0.22 µm syringe filter (Millipore) was conducted for final solution. Afterwards MeJa at the concentrations of 50 µM, 100 µM, and 200 µM with 3 different application durations (24h, 48h, and 72h) were supplied to cell suspension cultures (Krzyzanowska et al., 2012; Tanoori et al., 2015; Liu et al., 2018).

Regarding chitosan application; preparation of stock solution of chitosan from crab shells (Sigma) was prepared by dissolving in 0.1 HCl by heating gently and stirring continuously. The sterilization of the stock solution was provided by autoclaving. After this process, chitosan at the concentrations of 200 mg L^{-1}, 400 mg L^{-1} and 800 mg L^{-1} with 3 different application durations (24h, 48h, and 72h) were supplied to cell suspension cultures (Lim et al., 2013; Ahmed and Baig, 2014; Jiao et al., 2018). It was prepared 50% (v/v) ethanol solution for both elicitor treatments' control media.

To see the effects of elicitors on bioactive compounds first step is weighting up formed calli. To serve the purpose calli weight was scaled at the end of each application duration.

Reagents and solvents

For polyphenol analysis, evaluated chemicals were high purity. Methanol, acetonitrile, acetic acid (≥99.5%) were purchased from Isolab; mono- and di-caffeoylquinic acids were purchased as powder form namely were 3-*O*-caffeoylquinic acid (>98%) and 1,5-*O*-

dicaffeoylquinic acid (99%) (Toronto Research Chemicals); 4-*O*-caffeoylquinic acid (99%), 5-*O*-caffeoylquinic acid (99%), 1,3-*O*-dicaffeoylquinic acid (99%) (Cayman Chemical). Other phenolics were purchased by several firms, such as narirutin (99%), luteolin (99%), apigenin (99%) and apigenin 7-*O*-glucuronide (99%) (Cayman Chemical); *p*-coumaric acid (≥99%) and caffeic acid (≥98%) (Sigma-Aldrich); ferulic acid (99%) (J&K Scientific); luteolin 7-*O*-glucronide (87%) (Harbison Walker International). To conduct HPLC analysis, the Millipore Mill-Q Direct Q-3 ultrapure water system was used for ultrapure water.

Extraction of bioactive compounds and HPLC analysis

The extraction procedure and HPLC analysis of each bioactive compound were carried out as described in Pandino et al., (2010) with a few modifications. Each sample was grounded, weighed as 0.5±0.01 g, and then extracted with 80% ethanol solution (5.0 mL). These samples were vortex for 30 seconds and then kept overnight in a shaker with 250 rpm. At the end of this process, the samples were filtered with 0.45 µm PTFE filter, centrifuged at 10000 rpm for 10 minutes, then the supernatant was collected, 1 mL sample extract transferred to 2 mL vials and injected to HPLC-DAD instrument for bioactive compound analysis, the same procedure was repeated twice.

The Agilent 1100 HPLC instrument with a quaternary HPLC pump (G1311A), column oven (G1316A), auto sampler (G1313A), degasser (G1379A) and diode array detector (DAD) (G1315A) was used to conduct the bioactive compound analysis. To achieve the chromatographic separation of bioactive compounds, the Agilent Hypersil ODS 250 mm x 4.6 mm I.D., 5 µm particle size C18 column, operated at 28 ºC, was used.

For the determination of bioactive compounds quantitatively, the HPLC analysis method was adapted from Pandino et al., (2010); mobile phases were 5% acetic acid in water (mobile phase A) and acetonitrile (mobile phase B) at a flow rate 1.0 mL/min, the column oven temperature was 28 °C and the injection volume was 20 µL. The gradient started with 10% mobile phase B to reach 20% percent at 5 minutes, 40% mobile phase B at 45-minute, 100% mobile phase B at 55 minutes. HPLC-DAD chromatograms were determined by the limits of detection (LOD) and quantification (LOQ) values and the spectrum data were collected at 310 nm, 330 nm, and 280 nm. Each bioactive compound was identified based on the retention time (RT) and wavelength (λmax).

Statistical analysis

The experiment was carried out as a completely randomized factorial design with 3 replications. The data were analyzed with the statistical program JMP version 5.0.1 (SAS Institute Inc., Cary, NC, USA). It was performed ANOVA to determine the effects of cultivars and sub-cultures on certain bioactive components. Comparisons that obtained $P \leq 0.05$ were considered statistically significant. Additionally, correlation among all the obtained results was carried out through multivariate methods with the statistical program JMP version 5.0.1, with $P \leq 0.05$ as the threshold.

RESULTS and DISCUSSIONS

Callus and suspension culture

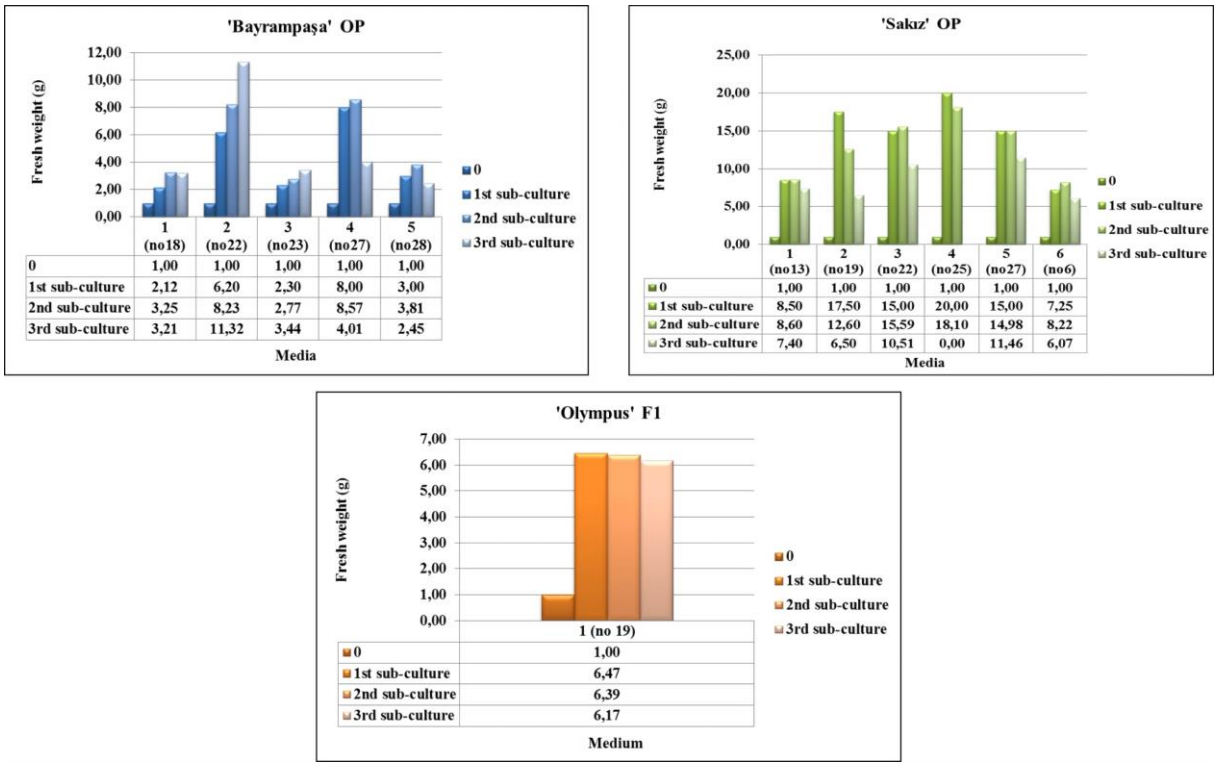
When experimental results were analyzed for callus and suspension culture, it was observed that each cultivar responded differently to all media combinations tested. Among the 29 callus induction media combinations, the most suitable media combinations for each cultivar were as down stated:

Five media combinations for Bayrampaşa OP cultivar: (1) 1.0 mg L^{-1} BAP + 1.0 mg L^{-1} NAA (medium no 18), (2) 0.1 mg L^{-1} KIN + 1.0 mg L^{-1} 2,4-D (medium no 22), (3) 0.1 mg L^{-1} KIN + 2.0 mg L^{-1} 2,4-D (medium no 23), (4) 1.0 mg L^{-1} KIN + 1.0 mg L^{-1} 2,4-D (medium no 27), (5) 1.0 mg L^{-1} KIN + 2.0 mg L^{-1} 2,4-D (medium no 28). Two media combinations (media no 22 and 23) among 5 stated media also increased biomass yield while others caused to a decrease (Fig. 1).

Six media combinations for Sakız OP cultivar: (1) 0.1 mg L^{-1} BAP + 1.0 mg L^{-1} NAA (medium no 13), (2) 1.0 mg L^{-1} BAP + 2.0 mg L^{-1} NAA (medium no 19), (3) 0.1 mg L^{-1} KIN + 1.0 mg L^{-1} 2,4-D (medium no 22), (4) 0.5 mg L^{-1} KIN + 1.0 mg L^{-1} 2,4-D (medium no 25), (5) 1.0 mg L^{-1} KIN + 1.0 mg L^{-1} 2,4-D (medium no 27), (6) 5.0 mg L^{-1} BAP + 5.0 mg L^{-1} NAA (medium no 6). Biomass yield got decreased in all media combinations (Fig. 1).

One medium combination for Olympus F_1 hybrid cultivar: (1) 1.0 mg L^{-1} BAP + 2.0 mg L^{-1} NAA (medium no 19). Unfortunately stated medium resulted with a decrease for biomass yield during each sub-culture for Olympus F_1 hybrid cultivar (Fig.1). Therefore Olympus F_1 hybrid cultivar was omitted for rest of the study.

Within the scope of callus cell suspension culture studies conducted under aseptic and controlled conditions, it is known that callus cultures are affected by many factors such as genotype, explant source, and composition of media. Compared to callus culture, cell suspension cultures require longer procedures but are considered a good source of uniform cells that enable scale production. Cell suspension cultures continue to differentiate with a short growth cycle under controlled conditions, thereby increasing the chances of repetition within and between experiments (Ngara et al., 2008; Abbas et al., 2018). Therefore, callus and cell suspension cultures can be conducted quickly using related *in vitro* culture techniques, and therefore accumulation of valuable bioactive components is enabled (Verpoorte et al., 2002; Abbas et al., 2018).



	1 (no18)	2 (no22)	3 (no23)	4 (no27)	5 (no28)
0	1,00	1,00	1,00	1,00	1,00
1st sub-culture	2,12	6,20	2,30	8,00	3,00
2nd sub-culture	3,25	8,23	2,77	8,57	3,81
3rd sub-culture	3,21	11,32	3,44	4,01	2,45

	1 (no13)	2 (no19)	3 (no22)	4 (no25)	5 (no27)	6 (no6)
0	1,00	1,00	1,00	1,00	1,00	1,00
1st sub-culture	8,50	17,50	15,00	20,00	15,00	7,25
2nd sub-culture	8,60	12,60	15,59	18,10	14,98	8,22
3rd sub-culture	7,40	6,50	10,51	0,00	11,46	6,07

	1 (no 19)
0	1,00
1st sub-culture	6,47
2nd sub-culture	6,39
3rd sub-culture	6,17

Figure 1. Biomass yield belongs to globe artichoke cultivars based on media combinations and number of sub-cultures

Şekil 1. Besi ortamı kombinasyonlarına ve alt kültür sayılarına göre enginar çeşitlerine ait biyokütle verimi

In many previous studies conducted on callus and suspension culture in different crops, it has been revealed that PGRs are so important factor for callogenesis. It has been also reported that another essential factor is that these PGRs should be added at appropriate concentrations and combinations to culture media (Ozsan and Onus, 2020b). It is possible to say that the PGRs combination of kinetin + 2,4-D is more effective than the BAP + NAA combination. Besides PGRs combination, the results revealed that concentrations of PGRs are also an essential factor for triggering calli formation. For obtaining calli, balance of auxin:cytokinin concentration based on cultivars was found to be important. It was observed that the most inducing concentrations of auxin:cytokinin 1:1 or 10:1. Therefore, obtained results from the current study were in accordance with previous studies (Ruta et al., 2013; Joshaghani et al., 2014; Abbas et al., 2018; Hesami and Daneshvar, 2018; Wani et al., 2018). If a general evaluation is made based on the findings of the present study, the accumulated bioactive compounds and their amounts varied according to cultivars and media combinations.

Elicitor treatments

Methyl jasmonate treatments belonging to Bayrampaşa OP cultivar ended with reducing on callus weights and the maximum change was determined when 200 µM MeJa was applied for 24 hours. However, the least loss of callus weight was detected in the application of 200 µM MeJa for 48 hours. When MeJa applications were evaluated in the Sakız OP cultivar, there was not as much loss in callus weights as in Bayrampaşa. However considering that most callus fresh weight losses took place in control media, it might be assumed that MeJa application prevented heavy callus weight loss for both cultivars.

Considering the results obtained in the observations made after chitosan application, decreases in callus fresh weights were noticeable in both cultivars.

Tanoori et al., (2015), who applied 200 µM MeJa to callus cultures, reported that with this application dose maximum phenolic acid accumulation took place and the increase rate was 3.96 times higher than the control group. The same researchers also reported that they achieved the highest flavonoid accumulation with a 100 µM MeJa application. Regarding chitosan application Ahmad et al., (2019) reported that different doses of chitosan applied to cell cultures in *Linum usitatissimum* L. (flax) had a positive effect on total phenolic accumulation, and the application increased their antioxidant activity by 1.3 times more than the control group. Since there was no increase on calli weight for both cultivars after elicitor applications, it was not possible to get enough amount of calli to see the effect of elicitor treatments on bioactive component contents. It is considered that cultivars may respond

differently to each elicitor type, and it is thought that the quality of elicitors, application doses and durations important factors.

In a study conducted on strawberries, it was shown that the production of anthocyanins and other metabolites caused pectinase consumption in cell cultures. Researchers concluded that there was a link between the formation of cell clusters and bioactive compounds (Edahiro and Seki, 2006). Therefore, the view arises that cell size can regulate bioactive compounds accumulation. Although no research was conducted on cell sizes in the present study, it could be speculated that MeJa and chitosan elicitor applications did not have any effect on the formation of cell clusters in both artichoke cultivars.

Evaluating of bioactive metabolites

Results of HPLC analysis regarding bioactive components revealed that there were statistically significant differences among cultivars and media combinations. Based on the results of the present study it is possible to say that cultivars respond differently to the content of media composition and the content of each media composition have different effects on different bioactive compound accumulation.

Regarding evaluated bioactive components, 3-*O*-caffeoylquinic acid, 4-*O*-caffeoylquinic acid, 5-*O*-caffeoylquinic acid, and 1,3-*O*-dicaffeoylquinic were determined in both OP cultivars, while caffeic acid, ferulic acid and luteolin 7-*O*-glucuronide were detected in only Sakız OP cultivar. Other bioactive components were detected in neither Bayrampaşa OP nor Sakız OP cultivars.

Accordingly, when suspension cultures of the Bayrampaşa OP cultivar were evaluated, two of the important bioactive components namely, 3-*O*-caffeoylquinic acid (227.43 mg kg^{-1}), and 5-*O*-caffeoylquinic acid (106.42 mg kg^{-1}), were detected in the medium 27 (1.0 mg L^{-1} kinetin + 1.0 mg L^{-1} 2,4-D). The other important bioactive components namely, 4-*O*-caffeoylquinic acid (108.10 mg kg^{-1}) and 1,3-*O*-dicaffeoylquinic acid (5.92 mg kg^{-1}), were the highest in the medium 22 (0.1 mg L^{-1} kinetin + 1.0 mg L^{-1} 2,4-D) (Table 1).

Table 1. Bioactive compounds and their values for Bayrampaşa OP cultivar based on media combination
Çizelge 1. Besi ortamı bazında Bayrampaşa çeşidi için biyoaktif bileşenler ve değerleri

	Media numbers				
Bioactive compounds	**1 (no 18)**	**2 (no 22)**	**3 (no 23)**	**4 (no 27)**	**5 (no 28)**
3-*O*-CQ	8.19^{d}	117.56^{b}	3.20^{e}	227.43^{a}	20.40^{c}
LSD value	1.82				
4-*O*-CQ	0.0^{b}	108.10^{a}	0.0^{b}	0.0^{b}	0.0^{b}
LSD value	0.59				
5-*O*-CQ	0.0^{d}	0.0^{b}	24.42^{c}	106.42^{a}	38.44^{b}
LSD value	1.64				
1,3-*O*-diCQ	0.0^{b}	5.92^{a}	0.0^{b}	0.0^{b}	0.0^{b}
LSD value	0.82				

Different letters in the same column and row show that the mean difference is a statistically significant difference at P≤0.05 level.
3-*O*-CQ: 3-*O*-caffeoylquinic acid, 4-*O*-CQ: 4-*O*-caffeoylquinic acid, 5-*O*-CQ: 5-*O*-caffeoylquinic acid, 1,3-*O*-diCQ: 1,3-*O*-dicaffeoylquinic acid.

When the Sakız OP cultivar was evaluated regarding suspension cultures, the highest values of 3-*O*-caffeoylquinic acid (171.67 mg kg^{-1}), 4-*O*-caffeoylquinic acid (138.21 mg kg^{-1}), 5-*O*-caffeoylquinic acid (12.99 mg kg^{-1}), and caffeic acid (38.50 mg kg^{-1}) were obtained in the medium 13 (0.1 mg L^{-1} BAP + 1.0 mg L^{-1} NAA). In addition, the other important bioactive component 1,3-*O*-dicaffeoylquinic acid (78.27 mg kg^{-1}) was detected in the medium 6 (5.0 mg L^{-1} BAP + 5.0 mg L^{-1} NAA). The other important bioactive components namely, ferulic acid (36.14 mg kg^{-1}) and luteolin 7-*O*-glucuronide (56.30 mg kg^{-1}), were obtained in the medium 27 (1.0 mg L^{-1} kinetin + 1.0 mg L^{-1} 2,4-D) (Table 2).

Regarding the media combinations media no 22 and 27 were common both for Bayrampaşa and Sakız OP cultivars. Results revealed that both cultivars responded differently to each media combination as 3-*O*-caffeoylquinic acid, 4-*O*-caffeoylquinic acid, and 5-*O*-caffeoylquinic acid came into prominence in Bayrampaşa, while 1,3-*O*-dicaffeoylquinic acid, luteolin 7-*O*-glucuronide, caffeic acid, ferulic acid were determined at high amounts in Sakız (Table 3).

The study conducted by Pandino et al., (2017) is accepted as one of the first studies on the accumulation of phytochemicals through cell suspension cultures in artichoke, and therefore comparison in the literature is limited. Pandino et al., (2017) reported that they detected the highest accumulation of phenolic substances on the 5th day of suspension culture in their study in artichoke, and this amount decreased by about 26% on the 10th day of culture. As a possible cause of this situation, they showed that the cells were

in the rapid growth phase in this process. Since there was also a decrease on the level of bioactive compounds at the end of the suspension culture studies in the present study, the cells of callus suspension cultures could be also at rapid growth phase, too.

Table 2. Bioactive compounds and their values for Sakız OP cultivar based on media combination
Çizelge 2. Besi ortamı bazında Sakız çeşidi için biyoaktif bileşenler ve değerleri

	Media numbers					
Bioactive compounds	**1 (no 13)**	**2 (no 19)**	**3 (no 22)**	**4 (no 25)**	**5 (no 27)**	**6 (no 6)**
3-*O*-CQ	171.67^{a}	89.45^{b}	78.17^{c}	57.83^{de}	56.06^{e}	60.31^{d}
LSD value	2.88					
4-*O*-CQ	138.21^{a}	54.20^{d}	67.34^{b}	60.61^{c}	29.35^{f}	40.48^{e}
LSD value	2.73					
5-*O*-CQ	12.99^{a}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}
LSD value	0.31					
1,3-*O*-diCQ	30.13^{c}	0^{d}	0^{d}	0^{d}	52.22^{b}	78.27^{a}
LSD value	1.05					
Caffeic acid	38.50^{a}	0^{e}	13.26^{c}	0^{e}	8.85^{d}	21.76^{b}
LSD value	0.84					
Ferulic acid	12.41^{c}	19.23^{b}	0^{e}	6.22^{d}	36.14^{a}	0^{e}
LSD value	0.75					
Lut. 7-*O*-gluc.	0^{c}	18.58^{b}	0^{c}	0^{c}	56.30^{a}	0^{c}
LSD value	0.97					

Different letters in the same column and row show that the mean difference is a statistically significant difference at P≤0.05 level.
3-*O*-CQ: 3-*O*-caffeoylquinic acid, 4-*O*-CQ: 4-*O*-caffeoylquinic acid, 5-*O*-CQ: 5-*O*-caffeoylquinic acid, 1,3-*O*-diCQ: 1,3-*O*-dicaffeoylquinic acid, Lut. 7-*O*-gluc.: luteolin 7-*O*-glucuronide.

Results from the current study revealed that extractions originating from cell suspension cultures had different bioactive compounds and the amount of each compounds are found to be at a different level. The reason for obtaining different bioactive compounds at different amounts can be attributed to several factors. For example Negrel and Javelle, (1995), reported that this could be caused by defense responses produced by enzymes acting on the cell wall, initiating the biosynthesis of various metabolites even in the same cell lines. It is argued that the production of certain compounds in cell cultures may not occur because their origin is undifferentiated cells, and as a consequence these cells lack specific metabolites and therefore require tissue-specific biosynthesis (Reis et al., 2018).

CONCLUSION

The present study aimed to increase bioactive components in three globe artichoke cultivars by using callus cell suspension cultures in various media combinations and by applying two elicitor treatments, namely methyl jasmonate and chitosan, at 3 different concentrations. The experimental results revealed that the Bayrampaşa OP and Sakız OP cultivars reacted differently from Olympus F_1 hybrid cultivar, and they were found to be more responsive to callus cell suspension culture based on the results of HPLC-DAD analysis. Regarding the media combinations media no 22 and 27 were common both for Bayrampaşa and Sakız OP cultivars. Results revealed that both cultivars responded differently to each media combination for different bioactive compounds. Regarding elicitor treatments, after two elicitors (MeJa and chitosan) application at different concentrations and durations decreases in callus fresh weights were noticeable in both cultivars. Although there was no increase on levels of bioactive compounds, it could not be obtained, it should be kept in mind that genotype, type of elicitor applied, concentration and duration are very important factors for elicitor application. Therefore different kinds of elicitors, application times, and concentrations should be used and tested for different artichoke cultivars, assuming that it is essential to pay attention to these factors for of the studies and ensuring their optimization for further artichoke callus cell suspension studies.

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Conflict of interest statement

The authors declared no conflict of interest.

Table 3. Comparison of bioactive compounds and their values for Bayrampaşa and Sakız OP cultivars based on media combination

Çizelge 3. Besi ortamı bazında Bayrampaşa ve Sakız çeşitlerinin biyoaktif bileşenler ve değerlerinin karşılaştırılması

3-*O*-CQ	**Bayrampaşa OP**		**Sakız OP**		**Mean values of media**
Media numbers	**1 (no 22)**	**2 (no 27)**	**1 (no 22)**	**2 (no 27)**	
Mean values of cultivars x media	117.56^{b}	227.43^{a}	78.17^{c}	56.06^{d}	**Medium 1 =** 97.86^{b}
Mean values of cultivars	172.50^{a}		67.11^{b}		**Medium 2 =** 141.75^{a}
LSD values	**LSD_{media} =** 1.53	**$LSD_{cultivars}$ =** 1.53	**$LSD_{cultivars\ x\ media}$ =** 2.17		
4-*O*-CQ	**Bayrampaşa OP**		**Sakız OP**		**Mean values of media**
Media numbers	**1 (no 22)**	**2 (no 27)**	**1 (no 22)**	**2 (no 27)**	
Mean values of cultivars x media	0^{b}	106.42^{a}	0^{b}	0^{b}	**Medium 1 =** 0^{b}
Mean values of cultivars	53.21^{a}		0^{b}		**Medium 2 =** 53.21^{a}
LSD values	**LSD_{media} =** 1.13	**$LSD_{cultivars}$ =** 1.13	**$LSD_{cultivars\ x\ media}$ =** 1.60		
5-*O*-CQ	**Bayrampaşa OP**		**Sakız OP**		**Mean values of media**
Media numbers	**1 (no 22)**	**2 (no 27)**	**1 (no 22)**	**2 (no 27)**	
Mean values of cultivars x media	0^{b}	106.42^{a}	0^{b}	0^{b}	**Medium 1 =** 0^{b}
Mean values of cultivars	53.21^{a}		0^{b}		**Medium 2 =** 53.21^{a}
LSD values	**LSD_{media} =** 1.06	**$LSD_{cultivars}$ =** 1.06	**$LSD_{cultivars\ x\ media}$ =** 1.50		
1,3-*O*-diCQ	**Bayrampaşa OP**		**Sakız OP**		**Mean values of media**
Media numbers	**1 (no 22)**	**2 (no 27)**	**1 (no 22)**	**2 (no 27)**	
Mean values of cultivars x media	5.92^{b}	0^{c}	0^{c}	52.22^{a}	**Medium 1 =** 2.96^{b}
Mean values of cultivars	2.96^{b}		26.11^{a}		**Medium 2 =** 26.11^{a}
LSD values	**LSD_{media} =** 0.95	**$LSD_{cultivars}$ =** 0.95	**$LSD_{cultivars\ x\ media}$ =** 1.35		
Caffeic acid	**Bayrampaşa OP**		**Sakız OP**		**Mean values of media**
Media numbers	**1 (no 22)**	**2 (no 27)**	**1 (no 22)**	**2 (no 27)**	
Mean values of cultivars x media	0^{c}	0^{c}	13.26^{a}	8.85^{b}	**Medium 1 =** 6.63^{a}
Mean values of cultivars	0^{b}		11.05^{a}		**Medium 2 =** 4.42^{b}
LSD values	**LSD_{media} =** 0.30	**$LSD_{cultivars}$ =** 0.30	**$LSD_{cultivars\ x\ media}$ =** 0.43		
Ferulic acid	**Bayrampaşa OP**		**Sakız OP**		**Mean values of media**
Media numbers	**1 (no 22)**	**2 (no 27)**	**1 (no 22)**	**2 (no 27)**	
Mean values of cultivars x media	0^{b}	0^{b}	0^{b}	36.14^{a}	**Medium 1 =** 0^{b}
Mean values of cultivars	0^{b}		18.07^{a}		**Medium 2 =** 18.07^{a}
LSD values	**LSD_{media} =** 0.49	**$LSD_{cultivars}$ =** 0.49	**$LSD_{cultivars\ x\ media}$ =** 0.70		
Lut. 7-*O*-gluc.	**Bayrampaşa OP**		**Sakız OP**		**Mean values of media**
Media numbers	**1 (no 22)**	**2 (no 27)**	**1 (no 22)**	**2 (no 27)**	
Mean values of cultivars x media	0^{b}	0^{b}	0^{b}	56.30^{a}	**Medium 1 =** 0^{b}
Mean values of cultivars	0^{b}		28.15^{a}		**Medium 2 =** 28.15^{a}
LSD values	**LSD_{media} =** 0.76	**$LSD_{cultivars}$ =** 0.76	**$LSD_{cultivars\ x\ media}$ =** 1.07		

Different letters in the same column and row show that the mean difference is a statistically significant difference at P≤0.05 level.

3-*O*-CQ: 3-*O*-caffeoylquinic acid, 4-*O*-CQ: 4-*O*-caffeoylquinic acid, 5-*O*-CQ: 5-*O*-caffeoylquinic acid, 1,3-*O*-diCQ: 1,3-*O*-dicaffeoylquinic acid, Lut. 7-*O*-gluc.: luteolin 7-*O*-glucuronide.

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