Research Article/Araştırma Makalesi

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MEDITERRANEAN AGRICULTURAL SCIENCES (2019) 32(2): 129-134
DOI: 10.29136/mediterranean.559194

A protocol on *in vitro* rooting of 'Bayrampaşa' artichoke (*Cynara scolymus* L.)

'Bayrampaşa' enginarının (*Cynara scolymus* L.) *in vitro* köklenmesi üzerine bir protokol

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ARTICLE INFO

Received 30 April 2019
Received in revised form 13 May 2019
Accepted 15 May 2019

***Keywords*:**

Asteraceae
Cynara scolymus L.
Plant tissue culture
Activated charcoal
Plant growth regulators

ABSTRACT

Artichoke, belongs to the family *Asteraceae* (*Compositae*), is cultivated in a very wide area in the world and regarded as a functional food due to the bioactive components. The propagation of artichoke via tissue culture offers considerable advantages. However one of the most important problem is *in vitro* rooting. Therefore, the main purpose of the present study was to develop an effective protocol on *in vitro* rooting of 'Bayrampaşa' cultivar which is one of the important local artichoke cultivar. To serve the purpose, after a successful micropropagation process, involving 3 subculture stages, well-developed plantlets were selected and rooted in different media compositions as ten different media including control group were used in present study. The differences between the media were provided by adding different growth regulators such as 10.0 mg l^{-1} indole-3-acetic acid (IAA), 6.0 mg l^{-1} indole butyric acid (IBA), 5.0 mg l^{-1} gibberellic acid (GA_3), 0.5 mg l^{-1} naphthaleneacetic acid (NAA) and activated charcoal (0, 1.0 and 2.0 g l^{-1}). Developments of plantlets were observed and recorded at 15 days intervals. According to the results obtained during the study, the medium containing IAA (10.0 mg l^{-1}) and 1.0 g l^{-1} of activated charcoal gave the best results in terms of rooting after micropropagation.

MAKALE BİLGİSİ

Alınış tarihi 30 Nisan 2019
Düzeltilme tarihi 13 Mayıs 2019
Kabul tarihi 15 Mayıs 2019

***Anahtar Kelimeler*:**

Asteraceae
Cynara scolymus L.
Bitki doku kültürü
Aktif karbon
Bitki büyüme düzenleyicileri

ÖZ

Asteraceae (*Compositae*) familyasında yer alan enginar, dünyada oldukça geniş bir alanda yetiştiriciliği yapılmakta ve içerdiği biyoaktif bileşenlerden dolayı fonksiyonel gıda olarak görülmektedir. Enginarın doku kültürü yoluyla üretimi dikkate değer avantajlar sunmaktadır. Öte yandan enginar doku kültürü çalışmalarında karşılaşılan en önemli problemlerden biri *in vitro* köklenmedir. Bu nedenle bu çalışmanın temel amacı enginarın *in vitro* köklenmesi üzerine etkili bir protokol geliştirmektir. Bu amaçla, 3 alt kültür aşamasını içeren başarılı bir mikroçoğaltım sürecinin ardından, iyi gelişme göstermiş olan bitkicikler seçilmiş ve çalışmada kullanılan 10 farklı besi ortamı kombinasyonunda (kontrol grubu dahil) köklendirilmiştir. Besi ortamları arasındaki farklılıklar, 10.0 mg l^{-1} indol asetik asit (IAA), 6.0 mg l^{-1} indol butirik asit (IBA), 5.0 mg l^{-1} gibberellik asit (GA_3), 0.5 mg l^{-1} naftalen asetik asit (NAA) gibi farklı büyüme düzenleyicileri ve aktif kömür (0, 1.0 ve 2.0 g l^{-1}) eklenerek sağlanmıştır. Bitkiciklerin gelişimleri 15 gün aralıklarla gözlemlenmiş ve kaydedilmiştir. Çalışmadan elde edilen verilere göre, mikroçoğaltım aşamasını takiben köklenme bakımından IAA (10.0 mg l^{-1}) ve 1.0 g l^{-1} aktif kömür içeren besi ortamı kombinasyonu en iyi sonuçları vermiştir.

1. Introduction

Artichoke (*Cynara scolymus* L.), belongs to the *Asteraceae* family which is represented by 1100 genus and approximately 25000 species in the world (Heywood 1978). Artichoke, one of the edible herbaceous perennial plants, originated and widely cultivated in the Mediterranean basin (Bianco 2005; Farag et al. 2013), has an importance amongst the others, thanks to its bioactive components. This precious plant has an ancient history of usage as food and folk medicine. Its capitula or head with fleshy leaves, called bracts, and receptacle comprise the edible parts of plant (Fratianni et al. 2007; Lattanzio et al. 2009; El Senousy et al. 2014; Falco et al. 2015). Even though for human nutrition is major usage area of artichoke, it is not the only function of artichoke (Lanteri and Portis 2008). Aside from edible parts have been enjoyed as food, the artichoke is

accepted as a well-known herbal medicine against several diseases. Artichoke is recognised as a functional food and its leaves are commonly used for the purpose of producing commercial herbal extracts (El Senousy et al. 2014). Especially the other parts such as external bracts, leaves and stems form approximately 60% of plant known as non-food industrial by-products (Llorach et al. 2002; Ruiz-Aceituno et al. 2016). In recent years high attention has been paid to 'old but gold' plant with new uses (Lattanzio et al. 2009). There are several studies reported in literature about artichoke's health-protective potential. Artichoke's nutritional and pharmacological characteristics are attributed to phenolic compounds mainly caffeoylquinic acid and its derivatives at high concentrations. Although at low amounts, other phenolics including flavonoids and anthocyanins that play a distinctive role in human health are defined, too (Llorach et al. 2002; Falco et al. 2015).

Traditionally, cultivation of artichoke is conducted with vegetative plant parts such as offshoots, ovoli, and stumps (Alp 2008; Ciancolini 2012; Falco et al. 2015). Especially agamic propagation via offshoots for multiplication is still favorable system among breeders (Calabrese 2009; Ciancolini 2012; El Boullani et al. 2012). In addition to these, gamic production is also possible for artichoke. However, due to issues of genetic variability and protandry, this production system is limited for obtaining hybrids. On the other hand, it is possible to face important disincentive factors such as low multiplication rates and diseases in traditional cultivation. On this point, we may overcome stated obstacles by using *in vitro* techniques. Particularly, *in vitro* artichoke studies that started with De Leo and Greco (1976) at the end of 1970s then at 1980s some studies (Ancora et al. 1981; Pecaut et al. 1983) provided significant results on *in vitro* propagation of artichoke (Ciancolini 2012). Especially meristem culture technique for micropropagation is so important to provide virus-free, healthy plant materials. Undoubtfully *in vitro* techniques are favorable for artichoke. However, at this point, we encounter another problem, *in vitro* root induction and root development. Up to now there were several studies conducted by using different strategies in the literature such as different types and concentrations of plant growth regulators (Harbaoui et al. 1982; Marras et al. 1985; Draoui et al. 1993; Iapichino 1996; Morzadec and Hourtmant 1997; Bedini et al. 2012; López-Pérez and Martínez 2015; Ercan 2016), various supplements like cyclodextrin, activated charcoal (Bigot and Foury 1984; Brutti et al. 2000) and pretreatments (darkness, two-phase) (López-Pérez and Martínez 2015) in order to improve *in vitro* artichoke rooting. At the end of all these studies, it was reported that root induction percentages might not be high enough and it might depend on genotypes (Benoit and Ducreux 1981; Morzadec and Hourmant 1997; López-Pérez and Martínez 2015). The difficulties in rooting artichokes *in vitro* are still one of the main hurdles to overcome in *in vitro* micropropagation.

Therefore, present study focused on the *in vitro* rooting of late artichoke cultivar 'Bayrampaşa', typically grown in Turkey, and is traditionally propagated by offshoots.

2. Materials and Methods

2.1. Plant Material

Artichoke plants of 'Bayrampaşa' were obtained from experimental fields of Akdeniz University, Antalya, Turkey. *In vitro* studies were conducted at Tissue Culture Laboratory of Department of Horticulture, Faculty of Agriculture, Akdeniz University and Tissue Culture Laboratory of Güney Agripark Agricultural Research and Development Center.

2.2. Culture establishment

For *in vitro* culture, selected growing mother plants were used. First of all, plants were washed with tap water and antibacterial soap (900 ml puried water + 100 ml antibacterial soap). Surface disinfection was provided by subsequent treatments in 40% and 20% of commercial bleach solution (4.5% sodium hypochlorite) for 15 min and 5 min respectively. After each treatment the explants were rinsed with sterilized distilled water. All sterilization processes were carried out in a laminar flow chamber. Meristems were excised and cultured into micropropagation medium containing Murashige and Skoog (MS) (Murashige and Skoog 1962) basal medium supplemented with 0.05 mg l^{-1} BA, 0.005 mg l^{-1} IBA, 3.0% sucrose and 6.0 g l^{-1} agar. Medium pH was adjusted to 5.8 and autoclaved (20 min at 121°C). At the end of the process, the cultures were maintained in a cultivation chamber at 25±1°C with 16/8 hours photoperiod.

2.3. Micropropagation

After successful sterilization of plant materials and induction of meristem culture, micropropagation studies were performed. Before root induction was initiated, three subsequent subcultures were conducted with 25-30 day intervals. Same culture room conditions, as above stated, were provided for all subcultures.

2.4. Improvement of root induction

In order to increase root induction, ten different media combinations (including control medium) were used (see Table 1). For creating differences between media, we used indole-3-acetic acid (IAA), indole butyric acid (IBA), gibberellic acid (GA_3) and naphthalene acetic acid (NAA) at various concentrations using, half strength MS medium. Hormone

Table 1. Media combinations used in the study.

Media Codes	Half strength MS (g l^{-1})	IAA (mg l^{-1})	IBA (mg l^{-1})	GA_3 (mg l^{-1})	NAA (mg l^{-1})	AC (g l^{-1})	Sucrose (%)	Agar (g l^{-1})
K_1	2.2	10.0	-	-	-	-	3.0	6.0
K_2	2.2	10.0	-	-	-	1.0	3.0	6.0
K_3	2.2	10.0	-	-	-	2.0	3.0	6.0
K_4	2.2	-	6.0	-	-	-	3.0	6.0
K_5	2.2	-	6.0	-	-	1.0	3.0	6.0
K_6	2.2	-	6.0	-	-	2.0	3.0	6.0
K_7	2.2	-	-	5.0	0.5	-	3.0	6.0
K_8	2.2	-	-	5.0	0.5	1.0	3.0	6.0
K_9	2.2	-	-	5.0	0.5	2.0	3.0	6.0
$K_{10\ (control)}$	2.2	-	-	-	-	-	3.0	6.0

concentrations were modified from previous studies (Ancora et al. 1981; Morzadec et al. 1997; Cavallaro et al. 2004; Tavazza et al. 2004; López-Pérez and Martínez 2015; Ercan 2016). Activated charcoal (AC) at 1.0 g l^{-1} and 2.0 g l^{-1} was also tested to see the effect of activated charcoal.

Healthy and approximately 5 - 7 cm explants after three subcultures were taken and transferred to rooting media. The experiments were conducted as three replicates and 40 explants were used for each replicate. Observations were performed 15-20 days after the explants were transferred in to the rooting media. Root development was evaluated in 4 different groups (≤ 3 cm, 3 – 5 cm, 5 – 7 cm, ≥ 7 cm) by measuring root lenghts. Effect of rooting media on plant development was assessed at 4 different development stages, namely percentage of *in vitro* root induction (%), greenhouse transfer of rooting plantlets (%), surviving plantlets in greenhouse (%), plants transferred from greenhouse to field after three weeks (%).

2.5. Plant acclimatization

After necessary recordings and classifications were done, rooted artichoke plantlets were transplanted from *in vitro* vessels to plastic trays, filled with the mixture of peat: perlite (1:1), and placed in greenhouse conditions for 30-40 days. During first ten days, the trays were kept under a polyethylene tunnel. Polyethylene cover was removed gradually. Plants were twice watered daily with fog system. At the end of the greenhouse acclimatization survived plantlets were recorded. After three weeks, surviving but not well-developed plants were removed. After this elimination, the percentage of plants transferred from greenhouse to field conditions were recorded.

2.6. Statistical analysis

ANOVA with Duncan's multiple range tests was performed to analyze the impact of various plant growth regulators with/without activated charcoal and root growth levels at the beginning of root induction.

3. Results and Disscussion

In the present study, the percentages of root formation and further development after greenhouse acclimation have been evaluated, based on media combinations. The *in vitro* rooting phase is one of the most critical stages of artichoke micropropagation. Therefore, the media used should be optimized according to the genotypes (López-Pérez and Martínez 2015). In this sense, as is seen from Table 2, the best root formation was found in medium K_8 (½MS + 5.0 mg l^{-1} GA_3 + 0.5 mg l^{-1} NAA + 1.0 g l^{-1} AC), followed by medium K_2 (½MS + 10.0 mg l^{-1} IAA + 1.0 g l^{-1} AC). These two media gave better rooting performance over control medium (Figure 1). Although these two media include different growth regulators, they both have 1.0 g l^{-1} AC. Although same hormones have been used, media combination with 2.0 g l^{-1} AC, resulted in limited rooting. The obtained results are in agreement with previous studies conducted on artichoke rooting with AC (Klein and Bopp 1971; Bigot and Foury 1984).

On the other hand, IBA had poorer effect on *in vitro* rooting than other auxins used in present study. When the percentages of rooted plants transferred to greenhouse were examined, it was found that medium K_3 (½MS + 10.0 mg l^{-1} IAA + 2.0 g l^{-1} AC) was the most successful, followed by medium K_6 (½MS + 6.0 mg l^{-1} IBA + 2.0 g l^{-1} AC). Media with 2.0 g l^{-1} AC were better than others, while medium K_2 (½MS + 10.0 mg l^{-1} IAA + 1.0 g l^{-1} AC) was the best (Figure 2). Considering field transfer and survival, the best results were obtained from medium K_9 (½MS + 5.0 mg l^{-1} GA_3 + 0.5 mg l^{-1} NAA + 2.0 g l^{-1} AC), followed by medium K_3 (½MS + 10.0 mg l^{-1} IAA + 2.0 g l^{-1} AC) (Figure 3). Activated charcoal has "darkness effect" in nutrition media and this could be the reason for the positive effect and resulted with improvement on organogenesis, especially on rooting (Klein and Bopp 1971; Bigot and Foury 1984; López-Pérez and Martínez 2015).

In this study, when the root formation rates (Table 3) and greenhouse transplanting of artichoke plants (Table 4) were examined, there were no statistical differences between the media compositions, while there were statistical differences among root growth levels. According to the results obtained, the root growth level at 3 - 5 cm and 5 – 7 cm were more successful than others for transplanting to greenhouse (Table 4). As an improvement on Bayrampaşa *in vitro* rooting, while Ercan (2016) stated that the longest root length was 3.70 cm on 'Bayrampaşa', we obtained more than 7 cm length (Figure 1) in present study.

In general, one of the significant stages of *in vitro* studies is acclimatization. It was reported that the ratio of surviving artichoke plants ranged between 75-83%, depending on appropriate plant morphology and having a good quality rooting (Rossi and De Paoli 1992; Lucchesini et al. 2001; Bedini et al. 2012; López-Pérez and Martínez 2015). After 30–40 days in greenhouse conditions the data about surviving plantlets was

Table 2. General results of *in vitro* root induction (%), greenhouse transfer of rooting plantlets (%), surviving plantlets in greenhouse (%), plants transferred from greenhouse to field after three weeks (%).

Media Codes	*In vitro* root induction (%)	Greenhouse transfer of rooting plantlets (%)	Surviving plantlets in greenhouse (%)	Plants transferred from greenhouse to field after 3 weeks (%)
K_1	15.00	72.00	15.00	0
K_2	28.33	64.00	54.54	16.66
K_3	22.50	88.00	25.00	33.33
K_4	19.16	82.60	42.10	12.50
K_5	13.33	56.25	44.44	0
K_6	20.83	84.00	38.09	0
K_7	10.83	76.92	10.00	0
K_8	30.00	66.66	29.16	14.28
K_9	25.83	61.29	15.78	66.66
$K_{10\ (control)}$	26.66	78.12	28.00	0

Figure 1. *In vitro* rooted artichoke plantlets from various rooting media combinations and developmental days; a. Medium K_2 (½MS + 10.0 mg l^{-1} IAA + 1.0 g l^{-1} AC) – 20 days; b. Medium K_3 (½MS + 10.0 mg l^{-1} IAA + 2.0 g l^{-1} AC) – 30 days; c. Medium K_8 (½MS + 5.0 mg l^{-1} GA_3 + 0.5 mg l^{-1} NAA + 1.0 g l^{-1} AC) – 30 days; d. Medium K_4 (½MS + 6.0 mg l^{-1} IBA) – 35 days; e. Medium K_5 (½MS + 6.0 mg l^{-1} IBA + 1.0 g l^{-1} AC) – 35 days; f. Medium K_6 (½MS + 6.0 mg l^{-1} IBA + 2.0 g l^{-1} AC) – 35 days; g. Medium K_9 (½MS + 5.0 mg l^{-1} GA_3 + 0.5 mg l^{-1} NAA + 2.0 g l^{-1} AC) – 35 days; h. Medium K_1 (½MS + 10.0 mg l^{-1} IAA) – 50 days; i. Medium K_3 (½MS + 10.0 mg l^{-1} IAA + 2.0 g l^{-1} AC) – 50 days; j. Medium K_{10} (½MS) – 50 days (bar= 1.0 cm).

Figure 2. Acclimatization stage (after 30 days *in vitro* rooting); a. Medium K_2 (½MS + 10.0 mg l^{-1} IAA + 1.0 g l^{-1} AC); b. Medium K_4 (½MS + 6.0 mg l^{-1} IBA) (bar= 1.0 cm).

Figure 3. Greenhouse acclimatised plants before transferring to experimental field; a. Medium K_2 (½MS + 10.0 mg l^{-1} IAA + 1.0 g l^{-1} AC); b. Medium K_3 (½MS + 10.0 mg l^{-1} IAA + 2.0 g l^{-1} AC); c. Medium K_4 (½MS + 6.0 mg l^{-1} IBA); d. Medium K_8 (½MS + 5.0 mg l^{-1} GA_3 + 0.5 mg l^{-1} NAA + 1.0 g l^{-1} AC); e. Medium K_9 (½MS + 5.0 mg l^{-1} GA_3 + 0.5 mg l^{-1} NAA + 2.0 g l^{-1} AC) (bar= 1.0 cm).

recorded. When the surviving plantlets were examined in present study, statistical differences were found in terms of both media combinations and root growth levels. The best among the media combinations was found as medium K_2 (½MS + 10.0 mg l^{-1} IAA + 1.0 g l^{-1} AC), followed by medium K_8 (½MS + 5.0 mg l^{-1} GA_3 + 0.5 mg l^{-1} NAA + 1.0 g l^{-1} AC). When surviving plantlets in greenhouse were recorded according to their root development levels, it was found that plants with 5-7 cm and ≥ 7 cm root development were better than others (Table 5).

After 30–40 days acclimatization stage, surviving plantlets were maintained in greenhouse conditions for three weeks. As a result of observations made, it was determined that all surviving plants had rooting. However, as stated before, the morphology of the plants is also important. For this reason, plants that did not have proper plant morphology had not been transferred to field conditions even if they were well rooted. The plants transferred from greenhouse to field after three weeks (%) are given in Table 6.

Table 3. *In vitro* root induction (%).

	Root Growth Levels				Average of media
Media Codes	**≤ 3 cm**	**3 – 5 cm**	**5 – 7 cm**	**≥ 7 cm**	
K_1	7.50	2.50	3.33	1.66	3.75
K_2	5.83	10.83	9.16	2.50	7.083
K_3	8.33	6.66	3.33	4.16	5.625
K_4	9.16	5.00	2.50	2.50	4.792
K_5	4.16	3.33	2.50	3.33	3.333
K_6	12.50	4.16	4.16	0	5.208
K_7	5.83	5.00	0	0	2.708
K_8	18.33	6.66	4.16	0.83	7.500
K_9	11.6	4.16	2.50	7.50	6.458
$K_{10\ (control)}$	3.33	10.83	3.33	9.16	6.667
Average of root growth levels	8.667^{a}	5.917^{ba}	3.500^{b}	3.167^{b}	

Percentage values were subjected to statistical analysis. Different letters in the same column and rows indicate a statistically significant difference at *P ≤ 0.05. P_{Media}= 0.49, $P_{Root\ Growth\ Level}$= 0.0015, $P_{Media*Root\ Growth\ Level}$= 0.55.

Table 4. Greenhouse transfer of rooted plantlets (%).

	Root Growth Levels				Average of media
Media Codes	**≤ 3 cm**	**3 – 5 cm**	**5 – 7 cm**	**≥ 7 cm**	
K_1	55.55	22.22	100.00	33.33	52.78
K_2	55.55	61.11	57.14	66.67	60.12
K_3	61.11	88.89	66.67	66.67	70.83
K_4	50.00	66.67	66.67	33.33	54.17
K_5	33.33	83.33	50.00	16.67	45.83
K_6	86.67	100.00	100.00	0	71.67
K_7	50.00	100.00	0	0	37.50
K_8	27.78	100.00	100.00	33.33	65.28
K_9	20.20	38.89	66.67	66.67	48.11
$K_{10\ (control)}$	66.67	65.47	100.00	61.11	73.31
Average of root growth levels	50.68^{b}	72.65^{a}	70.71^{a}	37.77^{b}	

Percentage values were subjected to statistical analysis. Different letters in the same column and rows indicate a statistically significant difference at *P ≤ 0.05. P_{Media}= 0.276, $P_{Root\ Growth\ Level}$= 0.001, $P_{Media*Root\ Growth\ Level}$= 0.068.

Table 5. Surviving plantlets in greenhouse (%).

	Root Growth Levels				Average of media
Media Codes	**≤ 3 cm**	**3 – 5 cm**	**5 – 7 cm**	**≥ 7 cm**	
K_1	0	0	33.33	16.67	12.50^{bdc}
K_2	0	50.00	86.67	66.67	50.83^{a}
K_3	0	16.67	44.44	38.89	25.00^{bdac}
K_4	0	33.33	50.00	33.33	29.17^{bdac}
K_5	0	0	66.67	33.33	$25,00^{bdac}$
K_6	11.11	16.67	100.00	0	31.94^{bac}
K_7	0	16.67	0	0	4.17^{d}
K_8	0	13.33	88.89	33.33	33.89^{ba}
K_9	0	0	0	21.67	5.42^{dc}
$K_{10\ (control)}$	0	0	0	46.67	11.67^{bdc}
Average of root growth levels	1.11^{c}	14.66^{cb}	47.00^{a}	29.05^{b}	

Percentage values were subjected to statistical analysis. Different letters in the same column and rows indicate a statistically significant difference at *P ≤ 0.05. P_{Media}= 0.0042, $P_{Root\ Growth\ Level}$= 0.001, $P_{Media*Root\ Growth\ Level}$= 0.068.

Table 6. Plants transferred from greenhouse to field after three weeks (%).

	Root Growth Levels				Average of Media
Media Codes	**≤ 3 cm**	**3 – 5 cm**	**5 – 7 cm**	**≥ 7 cm**	
K_1	0	0	0	0	0
K_2	0	0	0	50.00	12.50
K_3	0	0	0	50.00	12.50
K_4	0	0	0	11.11	2.78
K_5	0	0	0	0	0
K_6	0	0	0	0	0
K_7	0	0	0	0	0
K_8	0	0	0	33.33	8.33
K_9	0	0	0	50.00	12.50
$K_{10\ (control)}$	0	0	0	0	0
Average of root growth levels	0^{b}	0^{b}	0^{b}	19.44^{a}	

Percentage values were subjected to statistical analysis. Different letters in the same column and rows indicate a statistically significant difference at *P ≤ 0.05. P_{Media}= 0.1720, $P_{Root\ Growth\ Level}$= 0.0001, $P_{Media*Root\ Growth\ Level}$= 0.0942.

4. Conclusion

This study aimed to increase the *in vitro* rooting potential of 'Bayrampaşa' artichoke cultivar. We also evaluated the root development as well as whole plant grow in terms of 4 different developmental stages. Experimental results revealed that AC and different kind of auxin types were essential factors for *in vitro* root development of 'Bayrampaşa' artichoke cultivar. It is thought that findings of present study may shed more light to further experiments on artichoke *in vitro* rooting.

References

Alp HA (2008) Enginarda Doku Kültürü Koşullarının İyileştirilmesine Yönelik Çalışmalar. Yüksek Lisans Tezi, Ege Üniversitesi Fen Bilimleri Enstitüsü, İzmir.

Ancora G, Belli-Donini ML, Cuozzo L (1981) Globe artichoke plants obtained from shoot apices through rapid *in vitro* micropropagation. Scientia Hortic. 14: 207-213.

Bedini L, Lucchesini M, Bertozzi F, Graifenberg A (2012) Plant Tissue Cultures from Four Tuscan Globe Artichoke Cultivars. Cent. Eur. J. Biol. 7: 680-689. doi: 10.2478/s11535-012-0064-x.

Benoit H, Ducreux G (1981) Etude de quelques aspects de la multiplication végétative *in vitro* de l'artichaut (*Cynara scolymus* L.). Agronomie 1: 225-230.

Bianco VV (2005) Present situation and future potential of artichoke in the Mediterranean basin. Acta Hort. 681: 39-55.

Bigot C, Foury C (1984) *In Vitro* Propagation of Globe Artichoke (*Cynara scolymus* L.) from Seedlings: Field Comparison of Some Clones with Their Parent Lines. Agronomie 4: 699-710.

Brutti C, Apostolo NM, Ferrarotti SA, LLOrente BE, Krymkiewicz N. (2000) Micropropagation of *Cynara scolymus* L. employing cyclodestrins to promote rhizogenesis. Sci. Hort. 83: 1-10.

Calabrese N (2009) AA.VV. Il carciofo e il cardo, coordinamento scientifico N. Calabrese. Collana Coltura&Cultura, Ed. Script Bologna, Impianto 168-171.

Cavallaro V, Castiglione V, Avola G, Finocchiaro E (2004) Influence of different substrates on the *in vitro* rhizogenesis process of early artichoke [*Cynara cardunculu*s L. subsp. *scolymus* (L.) Heg], Acta Hort. ISHS 660: 267-272.

Ciancolini A (2012) CHARACTERIZATION AND SELECTION OF GLOBE ARTICHOKE AND CARDOON GERMPLASM FOR BIOMASS, FOOD AND BIOCOMPOUND PRODUCTION. PhD thesis, Università degli Studi della Tuscia, Italy and Institut National Polytechnique de Toulouse, France.

De Leo P, Greco B (1976) New technique of artichoke propagation: *in vitro* culture of apical meristems [Nuova tecnica di propagazione del carciofo: coltura *in vitro* di meristemi apicali], In: Atti 2nd Congr. Int. Sul Carciofo, Bari. Ed. Minerva Medica Torino, 657-667.

Draoui N, Ghorbel A, Kchouk ME (1993) *In vitro* culture of globe artichoke (*Cynara scolymus* L.) in Tunisia: utilization of *vitro* methods in artichoke improvement. Agricoltura Mediterranea 123: 139–145.

El Boullani R, Elmoshli A, El Finti A, El Mousadik A, Serghini MA (2012) Improved *in vitro* Micropropagation of Artichoke (*Cynara cardunculus* var. *scolymus* L.). European Journal of Scientific Research 80: 430-436.

El Senousy AS, Farag MA, Al-Mahdy DA, Wessjohann LA (2014) Developmental changes in leaf phenolics composition from three artichoke cvs. (*Cynara scolymus*) as determined via UHPLC–MS and chemometrics. Phytochemistry 108: 67–76.

Ercan N (2016) Effects of Various Growth Regulators on *in vitro* Rooting of Globe Artichoke (*Cynara scolymus* L.). Journal of Agricultural Science and Technology A 6: 335-340. doi: 10.17265/2161-6256/2016.05.005.

Falco BD, Incerti G, Amato M, Lanzotti V (2015) Artichoke: botanical, agronomical, phytochemical, and pharmacological overview. Phytochem Rev 14: 993–1018. doi: 10.1007/s11101-015-9428-y.

Farag MA, El-Ahmady S, Alian F, Wessjohann LA (2013) Metabolomics driven analysis of artichoke leaf and its commercial products via UHPLC–q-TOF-MS. Phytochemistry 95: 177–187.

Fratianni F, Tucci M, De Palma M, Pepe R, Nazzaro F (2007) Polyphenolic composition in different parts of some cultivars of globe artichoke (*Cynara cardunculus* L. var. *scolymus* (L.) Fiori). Food Chemistry 104: 1282-1286.

Harbaoui Y, Smaijin G, Welvaert W, Debergh P (1982) Assainissement viral de l'artichaut (*Cynara scolimus* L.) par la culture *in vitro* d'apex méristématiques. Phytopathologie Mediterrané 21: 15–19 (in French).

Heywood VH (1978) Flowering Plants of the World. Oxford University Press, Oxford, London.

Iapichino G (1996) Micropropagation of Globe Artichoke (*Cynara scolymus* L.) from underground dormant buds (ovoli). *In Vitro* Cell. Dev. Biol. Plant 32: 249-252.

Klein B, Bopp M (1971) Effect of activated charcoal in agar on the culture of lower plants. Nature 230:474.

Lanteri S, Portis E (2008) Globe artichoke and Cardoon. In Vegetables I: *Asteraceae*, *Brassicaceae*, *Chenopodicaceae*, and *Cucurbitaceae*. Prohens J. And Nuez F. (Eds.) 428 Springer 49-74.

Lattanzio V, Kroon PA, Linsalata V, Cardinali A (2009) Globe artichoke: A functional food and a source of nutraceutical ingredients. Journal of Functional Foods 1: 131-144.

Llorach R, Espin JC, Tomás Barberán FA, Ferreres F (2002) Artichoke (*Cynara scolymus* L.) Byproducts as a Potential Source of Health-Promoting Antioxidant Phenolics. Journal of Agricultural and Food Chemistry 50: 3458-3464.

López-Pérez AJ, Martínez JA (2015) *In Vitro* Root Induction Improvement by Culture in Darkness for Different Globe Artichoke Cultivars. *In Vitro* Cell. Dev. Biol. Plant 51: 160-165. doi: 10.1007/s11627-015-9667-z.

Lucchesini M, Mensuali-Sodi A, Massai R, Gucci R (2001) Development of autotrophy and tolerance to acclimatization of Myrtus communis transplants cultured *in vitro* under different aeration. Biologia Plantarum 44: 167-174.

Marras F, Foddai A, Fiori M (1985) La selezione sanitaria del carciofo "*Spinoso Sardo*". Informatore fitopatológico 35: 47–50 (in Italian).

Morzadec JM, Hourmant A (1997) *In vitro* rooting improvement of globe artichoke (cv. *Camus de Bretagne*) by GA_3. Sci. Hortic. 72: 59-62.

Murashige T, Skoog FA (1962) A Revised Medium for a Rapid Growth and Bio Assays with Tobacco Tissues Cultures. Physiologia Plantarum 15: 473-97.

Pecaut P, Dumas R, De Vaulx R, Lot H (1983) Virus free clone of globe artichoke (*Cynara scolymus*) obtained after *in vitro* propagation. Acta Hort. 131: 303-309.

Rossi V, De Paoli G (1992) Micropropagation of Artichoke (*Cynara scolymus*), In: Bajaj Y.P.S. (Ed.), Biotechnology in Agriculture and Forestry Vol 19. High- Tech and Micropropagation III, Spinger-Verlag, Berlin Heidelberg, Germany.

Ruiz-Aceituno L, García-Sarrió MJ, Alonso-Rodriguez B, Ramos L, Sanz ML (2016) Extraction of bioactive carbohydrates from artichoke (*Cynara scolymus* L.) external bracts using microwave assisted extraction and pressurized liquid extraction. Food Chemistry 196: 1156–1162.

Tavazza R, Papacchioli V, Ancora G (2004) An improved medium for in vitro propagation of globe artichoke (*Cynara scolymus* L.) cv. Spinoso sardo, Acta Hort. 660: 91-97.