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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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 (GA₃), 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.

ÖZ
Asteraceae (Compositae) familyasında yer alan enginar, dünyada oldukça geniş bir alanda yetiştirildiği yapılmaktadır ve içeriğinde biyolojik bileşenlerden dolayı faydalısa da şifa olarak görülmektedir. Enginanın doku kültürününמסרisindeki yeterliliğinden dolayı önemli bir problem olarak ortaya çıkmaktadır. Bu çalışmanın temel amacı enginanın in vitro köklenmesi üzerine etkili bir protokol geliştirmektir. Bu amaçla, 3 alt kültür aşaması içeren başarılı bir mikroqrtalıtma sürecindeki iyi gelişme göstermiş olan bitkicikler seçilmiş ve çalışmada kullanılan 10 farklı ortam kombinasyonunda (kontrol grubu dahil) köklenirlerdir. Bu ortamlar arasındaki farklılıklar, 10.0 mg l⁻¹ indol asetik asit (IAA), 6.0 mg l⁻¹ indol butyrik asit (IBA), 5.0 mg l⁻¹ gibberelik asit (GA₃), 0.5 mg l⁻¹ naftalen asetik asit (NAA) gibi farklı büyüme düzenleyicileri ve aktiv kömür (0, 1.0 ve 2.0 g l⁻¹) eklenerek sağlanmıştır. Bitkiciklerin gelişmeleri 15 gün aralıklarıyla gözlenen ve kaydedilmiştir. Çalışmanın elde edilen verilerle göre, mikroqrtalıtma aşaması takiben köklenen bakımından IAA (10.0 mg l⁻¹) ve 1.0 g l⁻¹ aktiv kömür içeren ortam kombinasyonu iyi sonuçlar 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 (Fratianne 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 agamic propagation via offshoots for multiplicity is still a feasible 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. Undoubtedly 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 (Harbour et al. 1982; Marras et al. 1985; Draoui et al. 1993; Iapichino 1996; Morzadec and Hourmant 1997; Bedini et al. 2012; López-Pérez and Martinez 2015; Ercan 2016), various supplements like cyclodextrin, activated charcoal (Bigot and Foury 1984; Brutt et al. 2000) and pretreatments (darkness, two-phase) (López-Pérez and Martinez 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 Dureux 1981; Morzadec and Hourmant 1997; López-Pérez and Martinez 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 (300 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 (GA₃) 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) | GA₃ (mg l⁻¹) | NAA (mg l⁻¹) | AC (g l⁻¹) | Sucrose (%) | Agar (g l⁻¹) |
|-------------|--------------------------|-------------|-------------|--------------|--------------|------------|-------------|--------------|
| K₁          | 2.2                      | 10.0        | -           | -            | -            | -          | 3.0         | 6.0          |
| K₂          | 2.2                      | 10.0        | -           | -            | -            | -          | 3.0         | 6.0          |
| K₃          | 2.2                      | 10.0        | -           | -            | -            | -          | 3.0         | 6.0          |
| K₄          | 2.2                      | -           | 6.0         | -            | -            | -          | 3.0         | 6.0          |
| K₅          | 2.2                      | -           | 6.0         | -            | -            | -          | 3.0         | 6.0          |
| K₆          | 2.2                      | -           | 6.0         | -            | -            | -          | 3.0         | 6.0          |
| K₇          | 2.2                      | -           | -           | 5.0          | 0.5          | -          | 3.0         | 6.0          |
| K₈          | 2.2                      | -           | -           | 5.0          | 0.5          | 1.0        | 3.0         | 6.0          |
| K₉          | 2.2                      | -           | -           | 5.0          | 0.5          | 2.0        | 3.0         | 6.0          |
| K₁₀(control) | 2.2                      | -           | -           | -            | -            | -          | 3.0         | 6.0          |

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concentrations were modified from previous studies (Ancora et al. 1981; Morzadec et al. 1997; Cavallaro et al. 2004; López 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 lengths. 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 survival 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 Discussion

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\(_1\) (%MS + 5.0 mg l\(^{-1}\) IAA + 0.5 mg l\(^{-1}\) NAA + 1.0 g l\(^{-1}\) AC), followed by medium K\(_2\) (%MS + 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\(_1\) (%MS + 10.0 mg l\(^{-1}\) IAA + 2.0 g l\(^{-1}\) AC) was the most successful, followed by medium K\(_6\) (%MS + 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\(_5\) (%MS + 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\(_1\) (%MS + 5.0 mg l\(^{-1}\) GA\(_3\) + 0.5 mg l\(^{-1}\) NAA + 2.0 g l\(^{-1}\) AC), followed by medium K\(_1\) (%MS + 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 |

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recorded. When the surviving plantlets were examined in the 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 K₂ (½MS + 10.0 mg l⁻¹ IAA + 1.0 g l⁻¹ AC), followed by medium K₈ (½MS + 5.0 mg l⁻¹ GA₃ + 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 (%).

| Media Codes | ≤ 3 cm | 3–5 cm | 5–7 cm | ≥ 7 cm | Average of media |
|-------------|--------|--------|--------|--------|-----------------|
| K          | 7.50   | 2.50   | 3.33   | 1.66   | 3.75            |
| K          | 5.83   | 10.83  | 9.16   | 2.50   | 7.083           |
| K          | 8.33   | 6.66   | 3.33   | 4.16   | 5.625           |
| K          | 9.16   | 5.00   | 2.50   | 2.50   | 4.792           |
| K          | 4.16   | 3.33   | 2.50   | 3.33   | 3.333           |
| K          | 12.50  | 4.16   | 4.16   | 0      | 5.208           |
| K          | 5.83   | 5.00   | 0      | 0      | 2.708           |
| K          | 18.33  | 6.66   | 4.16   | 0.83   | 7.500           |
| K          | 11.6   | 4.16   | 2.50   | 7.50   | 6.458           |
| K          | 3.33   | 10.83  | 3.33   | 9.16   | 6.667           |

Average of root growth levels: 8.667* 5.917** 3.500* 3.167*

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 (%).

| Media Codes | ≤ 3 cm | 3–5 cm | 5–7 cm | ≥ 7 cm | Average of media |
|-------------|--------|--------|--------|--------|-----------------|
| K          | 55.55  | 22.62  | 100.00 | 33.33  | 52.78           |
| K          | 55.55  | 61.11  | 57.14  | 66.67  | 60.12           |
| K          | 61.11  | 88.89  | 66.67  | 66.67  | 70.83           |
| K          | 50.00  | 66.67  | 66.67  | 33.33  | 54.17           |
| K          | 33.33  | 83.33  | 50.00  | 16.67  | 45.83           |
| K          | 86.67  | 100.00 | 100.00 | 0      | 71.67           |
| K          | 50.00  | 100.00 | 0      | 0      | 37.50           |
| K          | 27.78  | 100.00 | 100.00 | 33.33  | 65.28           |
| K          | 20.20  | 38.89  | 66.67  | 66.67  | 48.11           |
| K          | 66.67  | 65.47  | 100.00 | 61.11  | 73.31           |

Average of root growth levels: 50.68 37.21 70.71

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 (%).

| Media Codes | ≤ 3 cm | 3–5 cm | 5–7 cm | ≥ 7 cm | Average of media |
|-------------|--------|--------|--------|--------|-----------------|
| K          | 0      | 0      | 33.33  | 16.67  | 12.50**         |
| K          | 0      | 50.00  | 86.67  | 66.67  | 50.83*          |
| K          | 0      | 16.67  | 44.44  | 38.89  | 25.00**         |
| K          | 0      | 33.33  | 50.00  | 33.33  | 29.179**        |
| K          | 0      | 0      | 66.67  | 33.33  | 25.00**         |
| K          | 11.11  | 16.67  | 100.00 | 0      | 31.94**         |
| K          | 0      | 16.67  | 0      | 0      | 4.17**          |
| K          | 0      | 13.33  | 88.89  | 33.33  | 33.89**         |
| K          | 0      | 0      | 21.67  | 5.42**  |
| K          | 0      | 0      | 46.67  | 11.67** |

Average of root growth levels: 1.11 14.66** 47.00** 29.05**

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 (%).

| Media Codes | ≤ 3 cm | 3–5 cm | 5–7 cm | ≥ 7 cm | Average of media |
|-------------|--------|--------|--------|--------|-----------------|
| K          | 0      | 0      | 0      | 50.00  | 12.50           |
| K          | 0      | 0      | 0      | 50.00  | 12.50           |
| K          | 0      | 0      | 0      | 11.11  | 2.78            |
| K          | 0      | 0      | 0      | 0      | 0               |
| K          | 0      | 0      | 0      | 0      | 0               |
| K          | 0      | 0      | 0      | 33.33  | 8.33            |
| K          | 0      | 0      | 0      | 50.00  | 12.50           |
| K          | 0      | 0      | 0      | 0      | 0               |

Average of root growth levels: 0.0 0.0 0.0 19.44**

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= 0.1270, 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.

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