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Effects of Thidiazuron and Activated Charcoal on *in vitro* Shoot Proliferation and Rooting of Myrtle (*Myrtus communis* L.)

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ABSTRACT

In this study, a micropropagation approach was developed for a commercially important myrtle clone ‘Aşı Mersin’ in Turkey. The effects of different concentrations of thidiazuron (TDZ), 6-benzylaminopurine (BAP) and naphthalene acetic acid (NAA) on shoot formation and the effect of activated charcoal on rooting were studied. The most of the shoots were obtained on Murashige and Skoog (MS) medium containing 0.3 mg L⁻¹ TDZ + 0.1 mg L⁻¹ NAA in the 1st subculture (3.8 shoot), and from MS with 0.3 mg L⁻¹ TDZ + 0.01 mg L⁻¹ NAA in the 2nd subculture (4.0 shoot). The explants were rooted in ½MS containing indole-3-butyric acid (IBA), NAA and activated charcoal (AC). IBA applications induced more rooting than NAA. The medium supplemented with 1.0 mg L⁻¹ IBA + 2.0 g L⁻¹ AC resulted in the highest rooting ratio (80%). Addition of AC into the medium resulted in slight increase in the rooting ratio, significant increase in shoot length, and reduced darkening in the rooting area. Acclimatization was successful for 86% of the rooted plants.

Keywords: Myrtle; Proliferation; Activated charcoal; Thidiazuron; Acclimatization

Thidiazuron ve Aktif Kömürün Mersinin (*Myrtus communis* L.) *in vitro* Sürgün Proliferasyonu ve Köklenmesi Üzerine Etkileri

ESER BİLGİSİ

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ÖZET

Çalışmada, Mersin bitkisinin ticari öneme sahip “Aşı Mersin” klonu için bir mikro çoğaltım yöntemi geliştirilmiştir. Bu amaçla farklı konsantrasyonlarda thidiazuron (TDZ), 6-benzil amino pürin (BAP) ve naftalin asetik asit’in (NAA) sürgün proliferasyonu ve aktif karbonun (AK) köklenme üzerine etkileri belirlenmiştir. En yüksek sürgün sayıları 1. alt kültürde 3.8 adet ile 0.3 mg L⁻¹ TDZ + 0.1 mg L⁻¹ NAA içeren Murashige ve Skoog (MS) ortamından elde edilirken, 2. alt kültürde 4.0 adet ile 0.3 mg L⁻¹ TDZ + 0.01 mg L⁻¹ NAA içeren MS ortamından elde edilmiştir. *In vitro* sürgünler indole-3-butyric acid (IBA), NAA ve aktif kömür içeren ½MS ortamında köklendirilmiştir. IBA uygulamalarının NAA

uygulamalarına göre köklenmeyi daha fazla uyardığı belirlenmiştir. İçinde 1.0 mg L⁻¹ IBA + 2.0 g L⁻¹ AC bulunan ½MS ortamı en yüksek köklenme oranını (% 80) vermiştir. Ortama AC eklenmesi köklenme oranını nispeten ve sürgün uzunluğunu önemli derecede arttırmış ve kök bölgesindeki kararmayı ise azaltmıştır. Köklenmiş bitkilerin % 86'sı dış koşullara başarılı bir şekilde alıştırmıştır.

Anahtar Kelimeler: Mersin; Proliferasyon; Aktif kömür; Thidiazuron; Dış koşullara alıştırma

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1. Introduction

Myrtle is included in the *Myrtus* species of *Myrtaceae* family which is a large family containing approximately 132 genera and 5671 species (Govaerts et al 2008). Most of them grow in the tropical and subtropical regions of South America and Australia. The Mediterranean myrtle (*Myrtus communis* L.) is economically the most important species among the evergreen myrtles (Baytop 1997). The fruits and leaves of myrtles are used for anti-genotoxic, anti-mutagenic, antiseptic, anti-inflammatory purposes and the treatment of internal and topical infections (Bonjar 2004; Hayder et al 2004). Its essential oils can be used for the production of natural medicines and its leaves can be consumed as tea (Flamini et al 2004; Ogur, 1994). The fruits and leaves of wild myrtles are picked and evaluated by the growers who grow the promising genotypes with superior fruit quality, and then sold at local bazaars in Turkey.

The widespread use of myrtle has resulted in an increase in its production. However, the information about its reproduction is limited. Klein et al (2000) stated that, application of indole-3-butyric acid (IBA) promoted rooting upto 70% on cuttings of 'Rehovot' and 'Tzfat' myrtle cultivars taken between December and March. There are also studies on *in vitro* propagation of myrtle. BAP was used as the cytokinin in all micropropagation studies (Nobre 1994; Parra & AmoMarco 1998). However, none of the studies investigated the effects of thidiazuron, another type of cytokinin, on the micropropagation of myrtle.

Several plant species including myrtle secrete phenolic substances during *in vitro* culture and these phenolic substances inhibit the development of the

plant (Ruffoni et al 2003; Yağcı Tüzün et al 2013). Activated charcoal (AC) has been widely used in the micropropagation studies that the secretion of phenolics is encountered. AC has also been used for seed germination, anther culture, somatic embryogenesis and *in vitro* rooting studies (Blanc et al 1999; Kitsaki et al 2004; Laura et al 2006). At the rooting stage, AC delays the disintegration of the auxin added into the medium and positively affects plant development by adsorbing the inhibitors such as phenolic substances and abscisic acid (Thomas 2008). The plant growth regulators such as IBA and naphthalene acetic acid (NAA) were successfully used for the rooting of micro-cuttings in myrtle (Nobre 1994; Parra & AmoMarco 1998). However, Ruffoni et al (2003) reported that the micro-shoots of myrtle released dark exudates from the explant base during *in vitro* culture. It is well known that phenolic substances secreted by plants cause the darkening of explants, the decrease in the rooting rate and the slowdown of development. Solving the problems regarding the micropropagation of myrtle may contribute to the plant production and availability.

In the present study, the effect of TDZ in MS (Murashige & Skoog 1962) medium supplemented with NAA on shoot proliferation of myrtle clone "Aşı Mersin" was investigated and compared with BAP. The effect of AC in combination with IBA or NAA on *in vitro* rooting was also investigated.

2. Material and Methods

Explants of myrtle clone 'Aşı Mersin' (*Myrtus communis* L.), a commercially important and widely grown clone in Turkey were used as plant material. The shoot tips (0.5-1 cm) from approximately 25

years old mature plants grown in Antalya, Turkey were used in the culture, and the culture was initiated in the first week of May as described by Scarpa et al (2000).

2.1. Induction of proliferation

For surface disinfection, the shoots were incubated in 70% ethanol for 1 min and then the explants were shaken in 3% sodium hypochlorite solution containing one or two drops of Tween-20 for 10 minutes, and washed three times with sterile distilled water for 5 minutes. MS medium (Murashige & Skoog 1962) containing 0.1 mg L⁻¹ GA₃, 6 g L⁻¹ agar, 30 g L⁻¹ sucrose and NAA (0.01 and 0.1 mg L⁻¹) as auxin supplemented with either TDZ (0.1, 0.3 and 0.5 mg L⁻¹) or BAP (0.5, 1.0 and 2.0 mg L⁻¹) as cytokinin were used to determine the optimal plant growth regulator combination for shoot proliferation. The MS medium without plant growth regulators served as control. The pH of the medium was adjusted to 5.7 before the addition of agar and sucrose. The media was autoclaved at 121 °C for 15 min and dispensed into erlenmeyer flasks (50 mL flask⁻¹). The explants were incubated in a growth chamber at 25 ± 1 °C under 16 hour photoperiod provided by cool-white fluorescent lamps (140-150 µmol m⁻² s⁻¹) at the micropropagation stage. The explants were sub-cultured twice for a period of 4 weeks, and shoot number and shoot length per explant were determined at the end of each subculture.

2.2. Induction of rooting

In order to root the *in vitro* shoots, the plantlets were cultured in ½ MS medium containing 6 g L⁻¹ agar and 20 g L⁻¹ sucrose, and 0, 0.5, 1.0, 2.0 and 4.0 mg L⁻¹ IBA or NAA alone or in combination with 2.0 g L⁻¹ AC. The explants were incubated for 1 week at 25 ± 1 °C in the dark, and then they were transferred into a growth chamber and incubated there for 3 weeks at 25 ± 1 °C under 16 hours photoperiod provided by cool-white fluorescent lamps (140-150 µmol m⁻² s⁻¹). At the end of 4 weeks, the rooting ratio (%), root number and shoot length of the explants were determined.

2.3. Acclimatization

For acclimatization studies, approximately 150 micro shoots were planted into the ½ MS medium containing 1.0 mg L⁻¹ IBA + 2.0 g L⁻¹ AC which provided the highest rooting. Then, 100 healthy plants with root lengths of 1 cm were acclimatized to outdoor conditions by planting into the pots containing a mixture of peat + perlite (2:1). The plants were covered with a polyethylene covering and the relative humidity of the atmosphere inside the covering was reduced slowly by opening holes every day. The coverings were removed after 10 days.

2.4. Statistical analysis

Completely randomized design was used in *in vitro* proliferation and rooting experiments with 3 replications and 15 plants per replication. The data were subjected to the analyses of variance (ANOVA) at P ≤ 0.05 using MINITAB statistical software (MINITAB Inc., UK). Means were separated by Duncan's multiple range test at P ≤ 0.05. The percent data were transformed into arcsin angle values prior to analyses.

3. Results and Discussion

3.1. Induction of proliferation

In the first subculture, 0.3 mg L⁻¹ TDZ + 0.1 mg L⁻¹ NAA treatment produced the highest number of shoots (P<0.05) while there was no difference among the others. In the second subculture, 0.3 mg L⁻¹ TDZ in combination with 0.1 or 0.01 mg L⁻¹ NAA were significantly better (P<0.05) for proliferation than the rest of the treatments with the exception of 0.5 mg L⁻¹ TDZ treatments (Table 1; Figure 1). Applications of BAP were not significantly different from the control in terms of shoot number. In general, shoot lengths did not differ significantly among the treatments. We found that TDZ was more effective than BAP for proliferation. There have been studies regarding the effects of BAP as a cytokinin on the micropropagation of myrtle. Grigoriadou and Leventakis (2000) obtained the best propagation (3 shoots per explant) from MS

medium containing 3 μM BAP + 0.3 μM GA₃ + 0.05 μM NAA in the micropropagation of myrtle. Moreover, Parra & Amo-Marco (1998) obtained better results with a total of 38.5 shoots per explant at the end of 8 weeks using 1 mg L⁻¹ BAP in MS medium. To date, the highest number of shoots per explant (34.8) at the end of a month's culture was reported by Scarpa et al (2000) who used MS medium supplemented with 2 mg L⁻¹ BAP + 0.2 mg L⁻¹ NAA which might be resulted from the use of a different genotype. At the micropropagation stages, cytokinins are one of the most important components affecting the success ratio. San et al (2009), reported that the medium with both TDZ and BAP was more effective in the propagation of jujube as compared to the media containing only TDZ or BAP alone. We used both BAP and TDZ as source of cytokinin in the experiments. However, we obtained better results from TDZ treatments as compared to BAP treatments (Table 1). To our knowledge, this is the first report on the effects of TDZ on *in vitro* propagation of myrtle.



Figure 1- *In vitro* proliferation of myrtle on MS medium containing 0.3 mg L⁻¹ TDZ + 0.1 mg L⁻¹ NAA

Şekil 1- Mersinin 0.3 mg L⁻¹ TDZ + 0.1 mg L⁻¹ NAA içeren MS ortamında *in vitro* çoğaltılması

3.2. Induction of rooting

The effects of IBA, NAA and AC (2.0 g L⁻¹) were determined in the rooting stage of the study. The treatment of 1.0 mg L⁻¹ IBA + 2.0 g L⁻¹ AC resulted in the highest (80%) rooting (Table 2, Figure 2a). There

Table 1- *In vitro* proliferation of myrtle on MS medium containing plant growth regulators

Çizelge 1- Mersinin farklı büyüme düzenleyici maddeleri içeren MS ortamında *in vitro* çoğaltılması

Treatments*	I. Subculture		II. Subculture	
	Shoot number per explant**	Shoot length (cm)***	Shoot number per explant**	Shoot length (cm)***
Control	1.27 b	1.42	1.00 c	1.35
0.1 mg L ⁻¹ TDZ + 0.01 mg L ⁻¹ NAA	2.53 b	1.65	2.38 b	1.68
0.1 mg L ⁻¹ TDZ + 0.1 mg L ⁻¹ NAA	1.95 b	1.85	1.67 bc	1.54
0.3 mg L ⁻¹ TDZ + 0.01 mg L ⁻¹ NAA	2.40 b	2.08	4.00 a	1.53
0.3 mg L ⁻¹ TDZ + 0.1 mg L ⁻¹ NAA	3.80 a	1.75	3.87 a	1.58
0.5 mg L ⁻¹ TDZ + 0.01 mg L ⁻¹ NAA	2.48 b	1.55	3.10 ab	1.65
0.5 mg L ⁻¹ TDZ + 0.1 mg L ⁻¹ NAA	2.52 b	1.62	3.05 ab	1.57
0.5 mg L ⁻¹ BAP + 0.01 mg L ⁻¹ NAA	2.35 b	1.57	1.60 bc	1.55
0.5 mg L ⁻¹ BAP + 0.1 mg L ⁻¹ NAA	1.67 b	1.59	1.07 bc	1.46
1.0 mg L ⁻¹ BAP + 0.01 mg L ⁻¹ NAA	2.27 b	1.53	1.73 bc	1.28
1.0 mg L ⁻¹ BAP + 0.1 mg L ⁻¹ NAA	2.00 b	1.61	1.40 bc	1.46
2.0 mg L ⁻¹ BAP + 0.01 mg L ⁻¹ NAA	2.13 b	1.29	1.73 bc	1.35
2.0 mg L ⁻¹ BAP + 0.1 mg L ⁻¹ NAA	1.53 b	1.62	1.20 bc	1.41

*, GA₃ at 0.1 mg L⁻¹ was added to all treatments; **, different letters in the same column indicate significant difference among the means at P < 0.05; ***, non-significant

were no significant differences among 0.5, 1.0 or 2 mg L⁻¹ IBA or 0.5, 1.0, 2.0 or 4.0 mg L⁻¹ IBA + 2.0 g L⁻¹ AC. In general, root numbers changed between 1.2 and 2.8 per explant, but differences among the treatments were insignificant. It is known that both genotype and growth regulators significantly affect rooting in myrtle. Ruffoni et al (2003) obtained 45-50% rooting in myrtle clones PA2, DCCOD1 and AR6 in which IAA and IBA were used. Hatzilazarou et al (2003) reported clonal differences in rooting that 96% of microshoots of myrtle clone-A rooted on WPM medium containing 0.5 mg L⁻¹ IBA while 100% of microshoots of myrtle clone-B rooted on the same medium but containing 1.0 mg L⁻¹ IAA. In support of our findings, a 70% rooting ratio was obtained from the MS medium containing 1 mg L⁻¹ IBA (Grigoriadou & Leventakis 2000). However, we have observed darkening on the explant's base and an unhealthy shoot development when shoots were rooted without AC (Figure 2b) possibly due to the release of dark exudates from the explant's base (Ruffoni et al 2003). With the use of AC, darkening appeared to be reduced (Figure 2c). Likewise, in

other species such as *Zingiber officinale* and *Taxus mairei*, addition of AC into the medium prevented the darkening on the explants (Chang et al 2001; Guo et al 2007). It is known that in some plants, certain physiological defects such as growth deficiency and darkening occur because of the excretion of certain inhibitors such as phenolics. Adding AC into the medium was reported to remove dangerous effects of the substances synthesized by plants by adsorbing (Thomas 2008). Previous studies reported that the use of AC either alone or in combination with auxin significantly stimulated rooting (Yang et al 2006) and reduced the callus formation (Agrawal et al 2002). However, we did not observe increased rooting percentage % (NSD), and callus formation on the rooting area of shoots in the present study (Table 2). Effects of AC on the rooting of the myrtle microshoots were not previously known.

We have also measured shoot lengths of the explants during *in vitro* rooting experiment. The data showed significant differences in shoot lengths of the explants which ranged from 1.6 to 4.2 cm (Table 2).

Table 2- The *in vitro* rooting of myrtle on ½ MS medium supplemented with IBA, NAA and 2 g L⁻¹ AC

Çizelge 2- Mersinin IBA, NAA ve 2 g L⁻¹ AC içeren ½ MS ortamında *in vitro* köklendirilmesi

Treatments	Rooting (%)*	Root number**	Shoot length (cm)*
½ MS Medium	0	1	1.8 c
½ MS Medium + 0.5 mg L ⁻¹ IBA	66.7	abc	2.1
½ MS Medium + 1.0 mg L ⁻¹ IBA	60.0	abc	2.2
½ MS Medium + 2.0 mg L ⁻¹ IBA	53.3	abcd	2.3
½ MS Medium + 4.0 mg L ⁻¹ IBA	13.3	gh	2.5
½ MS Medium + 0.5 mg L ⁻¹ NAA	13.3	gh	1.5
½ MS Medium + 1.0 mg L ⁻¹ NAA	26.7	defg	1.8
½ MS Medium + 2.0 mg L ⁻¹ NAA	20.0	efg	1.3
½ MS Medium + 4.0 mg L ⁻¹ NAA	20.0	fgh	1.2
½ MS Medium + 2.0 g L ⁻¹ AC	6.7	hi	2.1
½ MS Medium + 0.5 mg L ⁻¹ IBA + 2.0 g L ⁻¹ AC	66.7	abc	2.6
½ MS Medium + 1.0 mg L ⁻¹ IBA + 2.0 g L ⁻¹ AC	80.0	a	1.9
½ MS Medium + 2.0 mg L ⁻¹ IBA + 2.0 g L ⁻¹ AC	73.3	ab	2.0
½ MS Medium + 4.0 mg L ⁻¹ IBA + 2.0 g L ⁻¹ AC	53.3	abcd	1.5
½ MS Medium + 0.5 mg L ⁻¹ NAA + 2.0 g L ⁻¹ AC	46.7	bcde	1.3
½ MS Medium + 1.0 mg L ⁻¹ NAA + 2.0 g L ⁻¹ AC	40.0	cdef	1.5
½ MS Medium + 2.0 mg L ⁻¹ NAA + 2.0 g L ⁻¹ AC	46.7	bcde	1.8
½ MS Medium + 4.0 mg L ⁻¹ NAA + 2.0 g L ⁻¹ AC	40.0	cdef	2.2

* , different letters in the same column indicate significant difference among the means at P < 0.05; **, non-significant

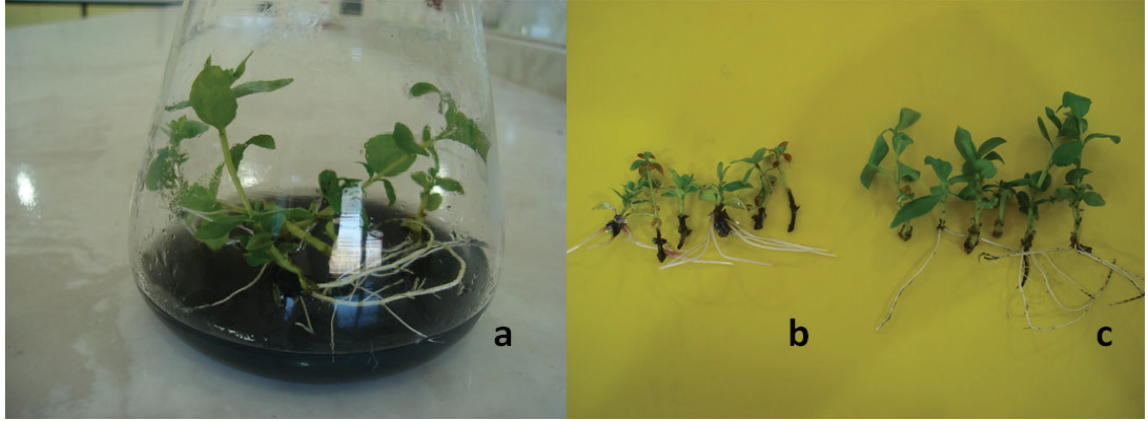


Figure 2- *In vitro* rooting of myrtles. a) microshoots rooted on $\frac{1}{2}$ MS medium containing 1 mg L^{-1} IBA + 2 g L^{-1} AC; b) unhealthy shoots and darkened area on the shoot base at treatments without AC; c) healthy shoots and little darkened area on the shoot base at treatments with 2 g L^{-1} AC

Şekil 2- Mersinin *in vitro* köklendirilmesi. a) 1 mg L^{-1} IBA + 2 g L^{-1} AC içeren $\frac{1}{2}$ MS ortamında köklendirilmiş mikroçelikler; b) AC içermeyen ortamlarda mikroçeliklerin köklenme bölgesinde kararma ve sağlıklı sürgünler; c) 2 g L^{-1} AC içeren ortamlarda köklenme bölgesindeki çok az kararma ve sağlıklı sürgünler

The highest shoot length (4.2 cm) was obtained with 1.0 mg L^{-1} IBA + 2.0 g L^{-1} AC treatment. Adding AC into the rooting medium significantly increased shoot length from 1.94 to 3.53 cm on average (Table 2, Figure 2b, c). Similarly, Datta et al (2006) and Stojicic et al (2012) reported the lengthening effect of AC on internodes in plants.

3.3. Acclimatization

The plants with approximately 1 cm root length were planted into a growth medium of peat + perlite (2:1) mixture and acclimatized. The results showed that rooted shoots were successfully acclimatized (86%) to outdoor conditions. Similar results were reported by Hatzilazarou et al (2003) who achieved over 97% of acclimatization in myrtles. One of the problems in myrtle micropropagation is the low success in acclimatization, because high doses of auxin in the rooting medium decrease the survival of plants at the stage of acclimatization (Welander 1983; Yepes & Aldwinckle 1994). However, we did not observe any problem in the acclimatization stage with using 1 mg L^{-1} IBA concentration. Another factor complicating the acclimatization of plants is the root length. When roots exceed 1 cm length, callus formation at the rooting area increases

and physiological defects develop on the roots (data not shown). Thus, our results showed that the plants should also be acclimatized to outdoor conditions as soon as they are rooted.

4. Conclusions

The medium containing TDZ was more effective in proliferation than BAP. For *in vitro* rooting of microshoots, $\frac{1}{2}$ MS medium supplemented with IBA was more effective than NAA. The addition of AC to the medium prevented the darkening of the explants, significantly increased shoot length and enhanced rooting. We suggest the use of MS medium with 0.3 mg L^{-1} TDZ + 0.1 or 0.01 mg L^{-1} NAA for the *in vitro* proliferation, and, $\frac{1}{2}$ MS medium containing 1.0 mg L^{-1} IBA + 2.0 g L^{-1} AC for rooting of myrtle clone (Aşı Mersin). We also suggest acclimatization of *in vitro* plants as soon as they are rooted.

References

- Agrawal V, Prakash S & Gupta S C (2002). Effective protocol for *in vitro* shoot production through nodal explants of *Simmondsia chinensis*. *Biologia Plantarum* **45**: 449–453

- Baytop T (1997). Türkçe bitki adları sözlüğü. Türk Dil Kurumu Yayınları, No: 578, Ankara, Turkey
- Blanc G, Michaux-Ferriere N, Teisson C, Lardet L & Carron M P (1999). Effects of carbohydrate addition on the induction of somatic embryogenesis in *Hevea brasiliensis*. *Plant Cell Tissue and Organ Culture* **56**: 17-23
- Bonjar G H S (2004). Antibacterial screening of plants used in Iranian folkloric medicine. *Fitoterapia* **75**: 231-235
- Chang S H, Ho C K, Chen Z Z & Tsay J Y (2001). Micropropagation of *Taxus mairei* from mature trees. *Plant Cell Reports* **20**: 496-502
- Datta M M, Majumder A & Jha S (2006). Organogenesis and plant regeneration in *Taxus wallichiana* (Zucc.). *Plant Cell Reports* **25**: 11-8
- Flamini G, Cioni P L, Morelli I, Maccioni S & Baldini R (2004). Phytochemical typologies in some populations of *Myrtus communis* L. on Caprione Promontory (East Liguria, Italy). *Food Chemistry* **85**: 599-604
- Govaerts R, Sobral M, Ashton P & Barrie F (2008). World Checklist of *Myrtaceae*. The University of Chicago Press Books, ISBN: 9781842463918
- Grigoriadou K & Leventakis N (2000). Preliminary study on large scale *in vitro* propagation of *Myrtus communis* L. more options. *Acta Horticulturae* **541**: 299-303
- Guo Y, Bai J & Zhang Z (2007). Plant regeneration from embryogenic suspension-derived protoplasts of ginger (*Zingiber officinale* Rosc.). *Plant Cell Tissue and Organ Culture* **89**: 151-157
- Hatzilazarou S, Grammaticos H, Economou A S, Rifaki N & Ralli P (2003). Rooting *in vitro* and acclimatization of *Myrtus communis* microcuttings. *Acta Horticulturae* **616**: 259-264
- Hayder N, Abdelwahed A, Kilani S, Ben Ammar R, Mahmoud A, Ghedira K & Chekir-Ghedira L (2004). Anti-genotoxic and free-radical scavenging activities of extracts from (Tunisian) *Myrtus communis*. *Mutation Research* **564**: 89-95
- Kitsaki C K, Zygouraki S, Ziobora M & Kintzios S (2004). *In vitro* germination, protocorm formation and plantlet development of mature versus immature seeds from several *Ophrys* species (*Orchidaceae*). *Plant Cell Reports* **23**: 284-290
- Klein J D, Cohen S & Hebbe Y (2000). Seasonal variation in rooting ability of myrtle (*Myrtus communis* L.) cuttings. *Scientia Horticulturae-Amsterdam* **83**: 71-76
- Laura M, Safaverdi G & Allavena A (2006). Androgenetic plants of *Anemone coronaria* derived through anther culture. *Plant Breeding* **125**: 629-634
- Minitab Inc. 2000. Meet Minitab: Release 13 for Windows ® 95/98/2000 and Windows NTTM. Minitab, USA
- Murashige T & Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* **15**: 473-497
- Nobre J (1994). *In vitro* shoot proliferation of *Myrtus communis* L. from field-grown plants. *Scientia Horticulturae-Amsterdam* **58**: 253-258
- Ogur R (1994). A Review about myrtle (*Myrtus communis* L.). *Çevre Dergisi* **10**: 21-25 (article in Turkish with an abstract in English)
- Para R & AmoMarco J B (1998). Factor affecting *in vitro* shoot proliferation of *Myrtus communis* L. a comparison of adult and seedling material. *In vitro Cellular and Developmental Biology-Pl* **34**: 104-107
- Ruffoni B, Airo M, Fascella G, Mascarella C, Zizzo G & Cervelli C (2003). Rooting and acclimatization of ornamental myrtle genotypes. *Acta Horticulturae* **616**: 255-258
- San B, Yildirim A N & Ecevit F M (2009). *In vitro* propagation of selected jujube (*Zizyphus jujuba* Mill.) genotypes, *33th Conference of the Agricultural Engineering*, Board of the Hungarian Academy of Sciences
- Scarpa G M, Milia M & Satta M (2000). The influence of growth regulators on proliferation and rooting of *in vitro* propagated myrtle. *Plant Cell Tissue and Organ Culture* **62**: 175-179
- Stojicic D, Janosevic D, Uzelac B, Cokesa V & Budimir S (2012). Micropropagation of *Pinus peuce*. *Biologia Plantarum* **56**: 362-364
- Thomas D T (2008). The role of activated charcoal in plant tissue culture. *Biotechnology Advances* **26**: 618-631
- Yağcı Tüzün C, Toker, M C & Toker G (2013). *Gentiana olivieri* Griseb.'in Kallus Kültürlerinin Kurulması ve Sekonder Metabolitlerin Araştırılması. *Tarım Bilimleri Dergisi - Journal of Agricultural Sciences*, **19**: 163-169
- Welander M (1983). *In vitro* rooting of the apple rootstock M26 in adult juvenile and growth phases and acclimatization of the plantlets. *Physiologia Plantarum* **58**: 231-238
- Yang L, Xu C J, Hu G B & Chen K S (2006). Direct shoot organogenesis and plant regeneration in *Fortunella crassifolia*. *Biologia Plantarum* **50**: 729-732
- Yepes L M & Aldwinckle H S (1994). Micropropagation of thirteen *Malus* cultivars and rootstock and effect of antibiotics on proliferation. *Plant Growth Regulation* **15**: 55-67