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Regeneration in *Origanum onites* L. by Plant Tissue Culture

Bitki Doku Kültürü ile Origanum onites' de Rejenerasyon

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Abstract

The aim of this study is *in vitro* regeneration in *Origanum onites* L. is a thyme species. The semi-solid MS medium which has been used successfully in rooting studies in many plant species in vitro, has been selected. After germinating *O. onites* seeds in hormone-free MS medium, hypocotyls, cotyledons, epicotyl, young primary leaves and apical meristem explants were cultured in an MS medium which contains a different concentration of kinetin, a plant growth regulator. After five days, it was observed that apical meristem explants directly shoot without forming calluses. Firstly, hypocotyl, cotyledon, apical meristem, first leaf and over-ground parts developed. Lastly, root development was observed, also was the least shoot formation in the medium containing 0.5 mg L^{-1} kinetin and the highest shoot formation was in the medium containing 1.5 mg L^{-1} kinetin.

Keywords: *In vitro, Origanum onites*, Organogenesis, Regeneration

Öz

Yaptığımız çalışmada amaç bir kekik türü olan *Origanum onites*'in *in vitro* ortamda köklenmesini sağlamaktır. *In vitro* ortam olarak birçok bitki türünde köklendirme çalışmalarında başarıyla kullanılmakta olan yarı- katı MS ortamı seçilmiştir. Hormonsuz MS ortamında *O. onites* tohumları çimlendirildikten sonra hipokotil, kotiledon, epikotil, genç primer yapraklar ve apikal meristem eksplantları bitki büyüme düzenleyicisi olan kinetinin farklı konsantrasyonunu içeren MS ortamına kültüre alınmıştır. Beş gün sonra apikal meristem explantlarının kallus oluşturmadan direkt olarak sürgün oluşturduğu gözlenmiştir. İlk olarak hipokotil, kotiledon, apikal meristem, ilk yaprak ve toprak üstü kısımlar gelişirken; son olarak kökün geliştiği, ayrıca en az sürgün oluşumunun 0.5 mg L^{-1} kinetin içeren ortamda en fazla sürgün oluşumunun ise 1.5 mg L^{-1} kinetin içeren ortamda olduğu gözlenmiştir.

Anahtar Kelimeler: *In vitro, Origanum onites*, Organogenez, Rejenerasyon

1. Introduction

Origanum onites (Lamiaceae) is a kind of thyme that grows naturally in Aegean and Mediterranean Regions in Turkey. This plant which has a very widespread use, and which is important in economy and medicine is added to food as a spice by people. In addition aerial parts are used in situations like gastric colds and headaches. It was found in studies which were conducted with essential oil that it has an analgesic effect. Linalool, carvacrol, and thymol in *O. onites* are effective in protecting cell membrane and they reduced the viability of some cancer cell types besides causing cell collapse (Bostancıoğlu et al. 2012). Because of the fact that it contains high levels of phenol and it has antibacterial, antiseptic, and antispasmodic effects, it is highly important in medicine (Oflaz et al. 2004). Other substances in *O. onites* are rosmarinic acid, carvacrol, thymol, γ-terpinene, γ-cymene, α-terpinene, and α-pinene (Ozkan et al. 2009).

Organogenesis is formation of organs such as leaves, shoots, or roots out of cells or tissue. It has two types of growth process depending on callus formation: direct organogenesis or indirect organogenesis. In direct organogenesis, cultured explants shoot without forming calluses while in indirect organogenesis, cultured explants shoot after callus formation. As organogenesis facilitates regenerating plants from cells and tissue, it enables production of plant species which are hard to reproduce in a generative way (Babaoğlu et al. 2001).

Kintzios (2002) used hypocotyls, cotyledons, roots, nodal segments, and leaves as sources of explants in his study with different kinds of *Origanum*. The researcher used MS (Murashige and Skoog 1962) and Gamborg B5 (1968) as

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culture mediums, 2,4-D (2,4 Dichlorophenoxyacetic acid) (10^{-7} M), BAP (6- Benzylamino purine) (10^{-6} M), IBA (Indole butyric acid) (10^{-6} M), and NAA (1- Naphthaleneacetic acid) (10^{-6} M) as plant growth regulators. Callus formation was observed in the MS medium with NAA (10^{-6} M) and BAP (10^{-6} M) and organogenesis from apical meristems was observed in the MS medium with BAP within 10-14 days.

Determining suitable culture conditions for *Epilobium parviflorum* and *O. onites* plants, Akbudak (2002) observed callus formation, plant regeneration, and other reactions by culturing explants taken from seedlings grown under sterile conditions in a half- strength MS essential nutrition medium which contains different combinations and concentrations of plant growth regulators. In an MS medium combined with 0.2 mg L^{-1} KIN (Kinetin)- 2 mg L^{-1} 2,4-D and 0.1 mg L^{-1}KIN- 1 mg L^{-1}2,4-D callus formation from cotyledon, petiole, and leaf explants was observed on dense flower willow herb. On Izmir thyme, in an MS medium containing 1.0 mg L^{-1} 2,4-D- 0.1 mg L^{-1} KIN and 2.0 mg L^{-1} 2,4-D- 0.1 mg L^{-1} KIN calluses were obtained from petiole and stem explants.

As studies have been conducted on shoot regeneration in other types of *Origanum,* especially *O. onites,* (Eguchi et al. 2000, Goleniowski et al. 2003, Sökmen et al. 2004, Fortunato et al. 2008, Oluk and Çakır 2009), there has been an increase in the importance of *O. onites* in time.

Since it is hard to reproduce *O. onites* in the Black Sea Region both because of the climate and the soil type it needs, in this study we aimed to observe especially root formation as well as other organs by using hypocotyls, cotyledons, epicotyl, young primary leaves and apical explants.

2. Material and Methods

2.1. Plant Material

O. onites seeds acquired from Ege University Faculty of Agriculture were sterilized in 15% commercial sodium hypochlorite (NaOCl) solution for 15 minutes. They were filtered through filter paper with the help of a funnel. In order to rinse the seeds, they were washed with distilled water three times. Sterilized in this way, the seeds were planted in jars (600 mm x 800 mm) containing hormone free MS mediums. They were checked for germination every day.

2.2. Culture Medium

After a germination process of 4 weeks, aseptic seedlings, hypocotyl (0.5 cm), epicotyl (0.5 cm), cotyledons (all), young primary leaves (all), the apical meristem (all) explants were cultured in petri dishes (100 mm x 100 mm) in a pH adjusted to 5.8 hormone injected MS medium with 30 g L^{-1}sucrose, 8 g L^{-1} agar. As the hormone, three different concentrations of KIN which are 0.5 mg L^{-1} (O1), 1 mg L^{-1} (O2) ve 1.5 mg L^{-1} (O3) were experimented. Using a total of 25 petri dishes, explants were cultured in 23.4 µmol m^{-2} s^{-1} (16/8 hour), in 25 ± 2°C temperature and callus formation, organogenesis, and other reactions were observed. The experiment was conducted three times in this way.

2.3. Statistical Analysis

"SPSS for Windows Ver. 19.0" (SPSS Inc., Chicago, IL., USA) was used for all the statistical analyses and one way analysis of variance (One Way ANOVA). The differences among means were compared by Duncan's multiple-range test (Duncan, 1955). All data were presented as mean ± standard error of three replicates.

3. Results and Discussion

O. onites seeds were germinated successfully in a hormone-free MS medium. First germination occurred after 4 days.

When hypocotyl, cotyledon, epicotyl, young primary leaves, apical meristem explants were taken from aseptic seedlings that were grown as a result of a 4 week long, germination process (Figure 1A) were cultured in a MS medium including different concentrations of KIN. It was observed that hypocotyl, cotyledon, epicotyl, young primary leaves were grown calluses a little 2 weeks later, but then it stopped. As for apical meristem explants it was observed that they didn't grow calluses, but directly shoot (Table 1). Those shoots firstly generated hypocotyl, cotyledon, epicotyl, young primary leaves, parts above the root, and lastly the root (Figure 1B). The first root formation was seen 3 weeks later (Figure 1C). Even though organ formation was observed in apical meristem explants in all hormone concentrations, the slowest (about after 4 weeks) was in the medium containing 0.5 mg L^{-1} KIN and the fastest (about after 3 weeks) was in the medium containing 1.5 mg L^{-1} KIN according to statistics (Table 1).

Researchers conducted experiments using different concentrations of different hormones (Kumari and Saradhi 1992, Kintzios 2002, Akbudak 2002). In previous studies on tissue culture, different concentrations of 2,4-D, NAA, BAP, IBA, KIN were tried as the hormone, in addition Gamborg B5 or MS were used as the culture medium (Kumari and Saradhi

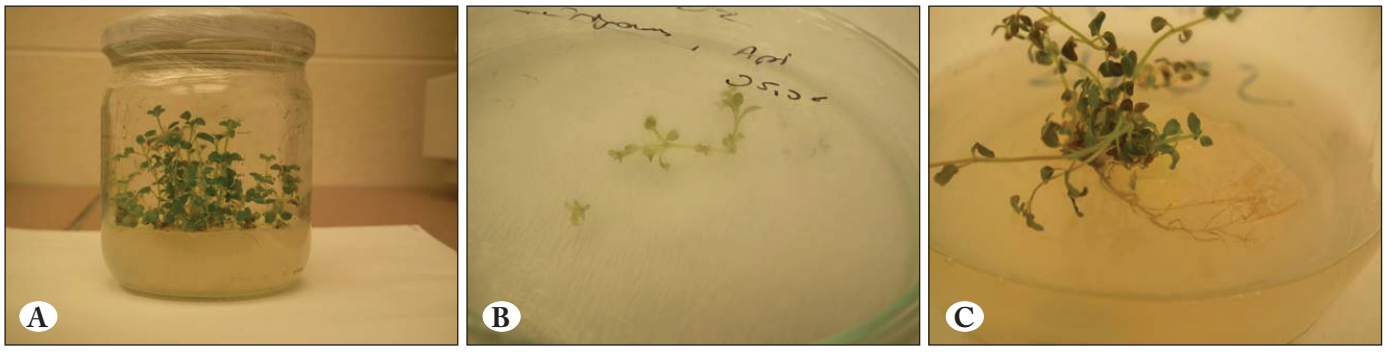

Figure 1. *Origanum onites:* **A)** After 4 week *O. onites* aseptic seedling; **B)** organogenesis in apical meristem explants; **C)** rooting and shoot regeneration.

Table 1. Direct organogenesis percent (%) from apical meristem explants which were taken from *O. onites* plant and shoot count percent (%) which forming from apical meristem explants.

Hormone Concentrations (KIN)	Direct Organogenesis from Apical Meristem Percent (%)	Shoot Count (%)
O1	100	56.04 ± 5.99a
O2	100	56.04 ± 5.99a
O3	100	81.56 ± 0.00b

Mean ± SE

1992; Kintzios 2002, Akbudak 2002). However, organogenesis did not take place in any of them.

Moreover, in previous studies (Kumari and Saradhi 1992, Kintzios 2002, Akbudak 2002) hypocotyls, petioles, cotyledons, young primary leaves, apical meristem explants, and roots were chosen as explants. However, in this study MS medium was chosen, different concentrations of kinetin hormone were used, and all the aerial parts were taken as sources of explants.

Although in previous tissue culture studies callus formation successfully took place, in this study it was not observed. Nevertheless, direct organogenesis took place from apical meristem explants. Thus, a new *O. onites* plant was obtained by direct organogenesis from apical meristem explants of *O. onites*.

This study was carried out using a similar culture medium and plant growth regulators as studies on shoot regeneration were made in other Origanum species (Eguchi et al. 2000, Goleniowski et al. 2003, Sökmen et al. 2004, Fortunato et al. 2008, Oluk and Çakır 2009).

O. onites is used in alternative medicine and to ease cold, stomachache, headache, toothache (Oflaz et al. 2004, Fakılı 2010). Since this plant is important in medicine, it needs to be grown at any time and easily which can be accomplished by plant tissue culture. This makes studies on organogenesis by plant tissue culture gain more importance.

4. References

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