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Original article

A Study on Developing a Sustainable In Vitro Propagation Method for Myrtle (*Myrtus communis* L.)

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Abstract

Myrtle (*Myrtus communis* L.), one of the typical natural plants of the Mediterranean Basin, has economic and ecological importance due to the essential oil obtained from its leaves, its fruits and its potential for use as an ornamental plant. However, factors such as habitat loss, overharvesting and low natural reproduction rates threaten the sustainability of myrtle populations. This research was carried out to determine the most appropriate nutrient medium combination for sustainable *in vitro* propagation of myrtle, to determine the optimum conditions for rooting of *in vitro* propagated plantlets and to acclimate the rooted plantlets to external conditions. MS + 1 mg/l BAP + 0.2 mg /l IBA medium gave the most successful results in terms of shoot number per explant and shoot length. The results obtained showed that myrtle clones selected for different purposes can be propagated in a short time by tissue culture.

Keywords: *Myrtus Communis, In Vitro* Propagation, Shoot Regeneration.

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INTRODUCTION

Myrtle (*Myrtus communis* L.) is a perennial, evergreen shrub, typically short but capable of growing to heights of 1–3 meters. It is naturally distributed across the Mediterranean region, the Middle East, temperate areas of North America, and Australia (Avcı and Bayram, 2008). As a characteristic plant of the Mediterranean basin, myrtle is found naturally in several regions of Turkey, including Adana, Antalya, Mersin, Çanakkale, Istanbul, Zonguldak, Sinop, Ordu, Trabzon, İzmir, Samsun, Muğla, and Hatay provinces (Avcı and Bayram, 2008). Myrtle holds significant economic and ecological importance due to its essential oils derived from leaves, fruits, and ornamental potential. It is widely used for reforestation after fire damage along the Mediterranean coastal zone and as an ornamental plant in European markets, particularly in bouquets as greenery. In Turkey, its fruits are in demand in local markets (Aka et al., 2020). Traditionally, myrtle has been recognized for its antimicrobial, antioxidant, and anti-inflammatory properties, contributing to its use in traditional medicine. It also plays a role in erosion control within natural habitats. In recent years, scientific studies on the bioactive components of myrtle have yielded promising results regarding its potential adaptation to modern pharmacology. However, factors such as habitat loss, overharvesting, and low natural propagation rates pose significant threats to the sustainability of myrtle populations. Despite its ecological and economic significance, studies on the propagation of myrtle remain limited.

Due to pressures on natural populations and slow propagation rates, it is essential to develop propagation and conservation strategies for *Myrtus communis* L. Traditional propagation methods often yield limited results, highlighting the potential of modern biotechnological approaches to address these challenges. Tissue culture techniques have emerged as effective tools for both propagating and preserving the genetic diversity of species like myrtle, which hold significant economic and ecological value. Grigoriadou and Leventakis (1999) investigated the applicability of tissue culture techniques for myrtle *in vitro* propagation and emphasized their role in selecting genotypes resistant to environmental stress factors. However, to ensure both biological and economic sustainability, optimization of these methods is necessary for their broader application.

This study aims to achieve the sustainable *in vitro* propagation of myrtle (*Myrtus communis* L.) by investigating various tissue culture methods and the effects of growth regulators. The research focuses on identifying the optimal culture medium combinations for sustainable *in vitro* propagation, rooting the *in vitro*-propagated plantlets, and determining the necessary conditions for acclimatizing the rooted plantlets to external environments. In this context, the development of an optimized and sustainable *in vitro* propagation protocol for myrtle is expected to contribute significantly to the scientific literature while offering valuable potential for agricultural and industrial applications.

MATERIAL and METHOD

This study was conducted in the laboratory and greenhouse of the Güney Agripark R&D Center. The plant material consisted of shoot tips collected on May 11, 2022, from the natural flora of Gazipaşa, Antalya (36°19'51.8"N 32°18'29.7"E). In tissue culture experiments, shoot tips were used as explants. For surface sterilization, the shoot tips were rinsed three times with sterile distilled water. They were then sterilized using 5% sodium hypochlorite (prepared from 30% commercial bleach) with a drop of Tween-20 for 15 minutes. Finally, the sterilized explants were rinsed three more times with sterile distilled water to ensure complete removal of the sterilizing agent.

The shoot regeneration experiment utilized MS (Murashige & Skoog, 1962) and WPM (Woody Plant Medium) as basal media. The media were supplemented with 2.19 mg/l glutamine, 30 g/l sucrose, 1 g/l polyvinylpyrrolidone (PVP), and 8 g/l agar. The pH of the medium was adjusted to 5.8 using 0.1 N NaOH or HCl. Different concentrations of BAP and IBA were added to the MS medium, while varying doses of 2iP were incorporated into the WPM medium (Table 1). The prepared media were sterilized in an autoclave at 121°C for 20 minutes. The sterilized shoot tip explants were then cultured on these media. The cultures were incubated in a climate-controlled chamber set at 23 ± 2°C with 3000 lux light intensity and a 16/8-hour light/dark photoperiod.

The experiment was designed with 10 different media and 5 replicates for each medium. Each 500 ml jar was considered one replicate, with 5 explants per jar. A total of 50 jars and 250 explants were used in the experiment.

Table 1. Basic media, hormone concentrations and combinations used in shoot regeneration experiments

Medium	Hormone Combinations
MS0 (Control)	-
MS	0,5 mg/l BAP + 0,1 mg/l IBA
MS	1,0 mg/l BAP + 0,2 mg/l IBA
MS	1,5 mg/l BAP + 0,3 mg/l IBA
MS	2,0 mg/l BAP + 0,4 mg/l IBA
WPM0 (Control)	-
WPM	1 mg/l 2IP
WPM	2 mg/l 2IP
WPM	5 mg/l 2IP
WPM	10 mg/l 2IP

In the rooting experiment, the MS medium was supplemented with different concentrations of NAA and IAA hormones (0, 0.5, 1.0, 1.5, and 2.0 mg/l) (Table 2). The experiment was conducted with 9 different media, each with 5 replicates. Each 500 ml jar was considered one replicate, containing 5 explants per jar. A total of 45 jars and 225 explants were used in this experiment.

Table 2. Hormone concentrations used in rooting experiments

Medium	Hormone combinations
MS0 (Control)	-
MS	0,5 mg/l NAA
MS	1,0 mg/l NAA
MS	1,5 mg/l NAA
MS	2,0 mg/l NAA
MS	0,5 mg/l IAA
MS	1,0 mg/l IAA
MS	1,5 mg/l IAA
MS	2,0 mg/l IAA

The *in vitro*-grown and rooted plants were gradually acclimatized to external conditions under plastic covers. The plants rooted in jars were thoroughly cleaned of agar using water and transferred to trays containing a 3:1 peat-to-perlite mixture in the greenhouse. The plants were then gradually acclimated to outdoor conditions under mini plastic tunnels.

RESULTS and DISCUSSION

Observations revealed that the highest shoot number (4 shoots per explant) and shoot length (6 cm) were obtained from the MS medium supplemented with 1 mg/l BAP + 0.2 mg/l IBA (Table 3, Figure 1). This combination ranked first in terms of shoot number per explant, followed by the WPM medium with 10 mg/l 2iP, which produced 3 shoots per explant. The lowest shoot number (1 shoot per explant) was observed in the control group. The effects of the basal media (MS and WPM) on shoot number did not show significant differences. However, regarding shoot length, the MS + 1 mg/l BAP + 0.2 mg/l IBA medium was followed by the MS + 1.5 mg/l BAP + 0.3 mg/l IBA medium, which produced shoots with an average length of 5 cm. When comparing basal media, the MS medium resulted in a higher average shoot length (4.2 cm) than the WPM medium (3.4 cm) (Table 3).

Khosh-Khui et al. (1984) achieved the highest shoot proliferation for *Myrtus communis* L. *in vitro* propagation using a ½ MS medium supplemented with 30 g/l sucrose, 1.5 mg/l BA, and 0.1 mg/l NAA. Similarly, Nobre (1994) reported the highest multiplication rate with an MS medium containing 2.0 mg/l BA and 0.05 mg/l NAA.

In a study conducted in Spain, nodal explants of myrtle were cultured in MS, Schenk and Hildebrandt (SH), and Heller (H) media (full strength, ½, and ¼ strength) supplemented with 4.4, 13.3, and 22.2 µM BA or 4.7, 14.0, and 23.2 mM kinetin. The optimal proliferation was observed in ¼ MS medium containing 4.4 µM BA (Parra and Amo-Marco, 1998). Grigoriadou and Leventakis (1999) successfully propagated myrtle *in vitro* using an MS medium supplemented with 3 µM BAP, 0.3 µM

GA3, 0.05 µM NAA, 20% sucrose, and 0.5% agar. These studies highlight the importance of medium composition and hormone combinations in optimizing *in vitro* propagation protocols for myrtle.

Rezaee and Kamali (2014) investigated the *in vitro* propagation of *Myrtus communis* L. using MS and WPM media supplemented with various concentrations of BA and IBA hormones. Their study concluded that WPM medium outperformed MS medium, with the best results obtained from WPM medium containing 4 mg/l BA and 0.1 mg/l IBA. In the study conducted by Aka et al. (2020), an MS medium supplemented with 1 mg/l BA and 30 g/l sucrose produced an average of 6.10 shoots per plantlet. A review of the literature indicates that MS medium is commonly used as the basal medium for shoot induction, with BAP as the most effective cytokinin. These above stated findings are consistent with the current study's results. However, while IBA was used as the auxin in this study, earlier studies have reported successful results with NAA for shoot induction. This suggests that both IBA and NAA can be effective, depending on the specific experimental conditions.

Table 3. Average shoot numbers and shoot lengths per explant obtained from starting (inititation) media

Medium	**Hormone Combinations**	**Number of Shoots/explant**	**Shoot Length (cm)**
MS0 (control)	-	1,0	3,0
MS	0,5 mg/l BAP + 0,1 mg/l IBA	2,0	3,0
MS	1,0 mg/l BAP + 0,2 mg/l IBA	4,0	6,0
MS	1,5 mg/l BAP + 0,3 mg/l IBA	2,0	5,0
MS	2,0 mg/l BAP + 0,4 mg/l IBA	2,0	4,0
Average		2,2	4,2
WPM0 (control)	-	1,0	3,0
WPM	1 mg/l 2IP	2,0	3,0
WPM	2 mg/l 2IP	2,0	3,0
WPM	5 mg/l 2IP	2,0	4,0
WPM	10 mg/l 2IP	3,0	4,0
Average		2,0	3,4

Figure 1. Formation and developments of shoots in MS + 1 mg/l BAP + 0,2 mg/l IBA media combination

According to the current results, the highest root number (4 roots) and root length (4 cm) were obtained in the MS medium supplemented with 1.0 mg/l IAA (Table 4, Figure 2). The average primary root number was 4, the average root length was 4 cm, and the plant height reached 5 cm. The rooting period was determined to range between 30 and 40 days (Table 4).

Table 4. Average root numbers and root lengths per explant obtained from rooting media

Medium	**Hormone Combinations**	**Number of Roots/explant**	**Root Length (cm)**
MS0 (control)	-	1,0	1,0
MS	0,5 mg/l NAA	2,0	1,0
MS	1,0 mg/l NAA	3,0	2,0
MS	1,5 mg/l NAA	3,0	2,0
MS	2,0 mg/l NAA	3,0	3,0
Average		2,4	1,8
MS0 (control)	-	1,0	1,0
MS	0,5 mg/l IAA	2,0	1,0
MS	1,0 mg/l IAA	4,0	4,0
MS	1,5 mg/l IAA	3,0	3,0
MS	2,0 mg/l IAA	2,0	2,0
Average		2,4	2,2

Grigoriadou and Leventakis (1999) achieved 70% rooting in *Myrtus communis* using an MS medium supplemented with 5.4 µM NAA and 5.0 µM IBA under *in vitro* conditions. Similarly, Ruffoni et al. (2010) propagated myrtle using an MS medium containing 0.5 mg/l BA and 0.2 mg/l IAA. The clones showed varying multiplication rates and rooting percentages, with higher rooting percentages

observed in media containing 0.5 mg/l IAA or IBA. Rezaee and Kamali (2014) tested different IBA concentrations (0, 1, 2, 3, 4, 5 mg/l) in MS media for the *in vitro* rooting of myrtle. The best results were obtained with 3 mg/l IBA.

As in the current study, the literature consistently reports successful rooting results using MS medium as the basal medium and IAA or IBA as the rooting hormones. These findings underline the efficacy of these auxins for optimizing *in vitro* rooting protocols for myrtle.

Figure 2. Root formation in MS + 1,0 mg/l IAA media combination

The *in vitro*-grown and rooted plants were gradually acclimatized to external conditions under plastic covers. A total of 95% of the plants successfully adapted to outdoor conditions and were subsequently transferred to normal greenhouse conditions for growth. The total duration from the initiation of the culture to the transfer of plants to greenhouse conditions was calculated to be 24 weeks.

Conclusion

The results of this study demonstrate that selected myrtle (*Myrtus communis*L.) clones can be rapidly propagated through tissue culture for various applications. The successful outcomes, particularly with low hormone concentrations, highlight the importance of sustainability in developing efficient and scalable *in vitro* propagation protocols.

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