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Research Article

Synthetic Seed Production and Cryopreservation for Myrtle (*Myrtus communis* L.) Genotypes

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ABSTRACT
This comprehensive study delves into synthetic seed production and cryopreservation for *Myrtus communis* L., presenting a novel approach to conserving plant genetic resources. Our research highlights shoot tips' encapsulation and subsequent conversion into plants following short-term (+4°C) and long-term (-196°C) preservation periods. The study rigorously compares the viability and efficiency of these cryopreserved seeds with conventional propagation methods. We extensively investigated various factors affecting the success of synthetic seed production, including explant selection, medium composition, and encapsulation techniques. Additionally, the impact of different preservation temperatures on synthetic seeds' viability and germination rates was a focal point. Our results demonstrate a significantly high conversion rate of synthetic seeds to plants post-cryopreservation, indicating the reliability of this method in germplasm conservation. The findings of this research are pivotal for conserving *Myrtus communis* L. genotypes and offer valuable insights and methodologies applicable to other plant species. This study marks a critical advancement in plant biotechnology and germplasm conservation, providing a sustainable solution for preserving valuable genetic resources in the face of escalating environmental challenges.
Keywords: Cryopreservation, Genetic Conservation, Myrtle, Plant Biotechnology, Synthetic Seeds[2]

Mersin (*Myrtus communis* L.) Genotipleri İçin Sentetik Tohum Üretimi ve Kriyoprezervasyon

ÖZ
Bu kapsamlı çalışma, *Myrtus communis* L. için sentetik tohum üretimi ve kriyoprezervasyon alanına derinlemesine bir bakış sunmakta ve bitki genetik kaynaklarının korunmasına yönelik yenilikçi bir yaklaşımı ortaya koymaktadır. Araştırmamız, sürgün uçlarının kapsülasyonunu ve kısa süreli (+4°C) ve uzun süreli (-196°C) koruma sürelerinin ardından bitkilere dönüşümünü vurgulamaktadır. Çalışma, kriyoprezerve edilmiş tohumların canlılığını ve verimliliğini geleneksel üretim yöntemleriyle karşılaştırmaktadır. Sentetik tohum üretiminin başarısını etkileyen çeşitli faktörler, özellikle eksplant seçimi, ortam bileşimi ve kapsülleme teknikleri üzerinde kapsamlı bir inceleme yapılmıştır. Ayrıca, farklı koruma sıcaklıklarının sentetik tohumların canlılık ve çimlenme oranları üzerindeki etkisi de çalışmanın odak noktalarından biri olmuştur. Sonuçlarımız, sentetik tohumların kriyoprezervasyondan sonra bitkilere yüksek oranda dönüşüm gösterdiğini, bu yöntemin tohum gen kaynaklarının korunmasındaki güvenilirliğini göstermektedir. Bu araştırmanın bulguları, *Myrtus communis* L. genotiplerinin korunması için hayati öneme sahip olup, diğer bitki türleri için de değerli içgörüler ve metodolojiler sunmaktadır. Bu çalışma, bitki biyoteknolojisi ve gen kaynaklarının korunması alanında kritik bir ilerleme olarak, artan çevresel zorluklar karşısında değerli genetik kaynakları korumanın sürdürülebilir bir çözümünü sağlamaktadır.
Anahtar Kelimeler: Kriyoprezervasyon, Genetik Koruma, Mersin, Bitki Biyoteknolojisi, Sentetik Tohumlar

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Introduction

Myrtle, also known as *Myrtus communis* L., is a characteristic plant of the Mediterranean region, comprising perennial, evergreen shrubs, or small trees. Myrtle is a member of the Myrtaceae family, which naturally grows in tropical and subtropical regions and includes about 100 genera and 3000 species (Rezaee and Kamali, 2014; Şimşek et al. 2023). Various parts of the myrtle plant (leaves, flowers, fruits) contain important components for the medical, food, liquor, and cosmetic industries, making it valuable for anti-genotoxic, anti-mutagenic, antiseptic, and anti-inflammatory purposes, as well as for treating internal and tropical infections (Serce et al., 2008). Myrtle essential oils are useful in pharmaceutical production, and its leaves can be consumed as tea (Flamini et al. 2004; Şimşek et al. 2022). Myrtle plants, ranging from 0.5 to 3 meters in height, are slow growing but long-lived, always remaining green as they do not shed their leaves in winter (Picci and Atzei, 1996). The leaves have an aromatic scent, and its fruits, typically blackish-purple or white, mature in late autumn (October-December), are edible, and have a sweet and astringent taste (Sumbul et al., 2011; Aleksic & Knezevic, 2014). The fruit is pollinated by insects and covered with a slight waxy layer, which helps bees recognize the plant. This multi-seeded fruit contains volatile oils, tannins, sugars, and organic acids (citric and malic acid). Myrtle leaves are rich in phenolic acids like caffeic, ellagic, and gallic acids and quercetin, catechin, and myricetin derivatives (Simsek et al., 2020).

Myrtle can be propagated through seeds, cuttings, and *in vitro* techniques. The totipotency of plant tissues - the ability to differentiate and form a new plant - is a significant feature in plant tissue culture, enabling rapid clonal propagation and the cultivation of disease-free plants. This method is increasingly important for conserving Plant genetic resources through *in situ* (natural habitat) or *ex situ* (outside natural habitat) methods (Grzegorczyk and Wysokińska 2011).
The concept of a "Gene Center" or "Center of Origin", where plants first appeared and evolved, is crucial in botany. Russian botanist Vavilov identified 8 such Gene Centers worldwide, with Turkey being significant within the Near East and Mediterranean basin (Tan, 1998). The conservation of plant biological diversity, known as "genetic erosion," is critical for protecting endangered plant species, forms, and varieties. Although *in vitro* conservation methods offer short-term storage, they are economically inefficient, require extensive space, and are prone to contamination and somaclonal variation. Cryopreservation, developed in the late 1900s, involving ultra-low temperatures (-196 ºC) in liquid nitrogen, provides a suitable method for long-term germplasm conservation. It slows down biological materials, making it applicable to various plant tissues and organs (Towill and Bajaj, 2002; Lambardi and De Carlo, 2003).

Recent advancements in cryopreservation have led to more straightforward, cost-effective techniques, such as one-step freezing based on vitrification, where cell vitrification prevents harmful intracellular ice formation by transforming the cytosol into a glassy, amorphous state (Engelmann, 2004). Vitrification can be chemically induced with high concentration cryoprotectants like PVS2 or physically through desiccation methods, followed by direct transfer to liquid nitrogen. After an hour in liquid nitrogen, samples are quickly thawed in a pre-heated water bath and transferred to a regeneration medium under standard culture conditions (Sakai et al., 1990).

This study explores the *in vitro* conservation possibilities for *M. communis* from selected genotypes in the Adana and Mersin regions, Türkiye, known for their superior characteristics. It evaluates the benefits of synthetic seed technology for micropropagation and short-term conservation of myrtle genotypes and examines the efficacy of cryopreservation for long-term conservation. The primary goal is to develop strategies for conserving the genetic resources of the myrtle plant. This includes bringing shoot tips of myrtle genotypes to the laboratory, sterilizing them, converting them into synthetic seeds with Na-alginate coating, and subjecting them to short-term conservation at +4°C for 45

days or long-term conservation via cryopreservation. The study aims to provide an innovative and effective strategy for conserving the genetic diversity of the myrtle plant. It demonstrates the potential of synthetic seed technology and cryopreservation as tools for sustainable conservation and transmission of myrtle plant genetic resources to future generations.

Material and Methods

Plant Material

This study used two genotypes of myrtle from Tarsus/Mersin, Türkiye, one bearing white and the other black fruits. The plant materials were obtained in September 2022. These particular genotypes were chosen for their visually apparent high yield, large fruit size, and robust and healthy plant structure.

Method

Sterilization of Shoot Tips

The shoot tips from the genotypes used in the study were obtained and brought to the laboratory. Before culture initiation, the shoot tips were sterilized. To this end, the shoot tips were first washed under tap water for 10 minutes. In a sterile cabinet, the shoot tips were immersed in 70% ethyl alcohol for 3-4 minutes, followed by a rinse with sterile distilled water. Subsequently, they were treated with a 20% sodium hypochlorite solution for 15 minutes. The shoot tips were rinsed three times with sterile distilled water inside the sterile cabinet to remove the sterilant.

Culture Medium and Establishment of Micropropagation

After sterilization, the shoot tips were placed in MS (Murashige and Skoog, 1962) nutrient medium containing 1mg/L BAP (Benzyl Amino Purine), 30 g/l sucrose, and 7.5 g/l agar. The pH of the nutrient medium was adjusted to 5.7, and then the medium was poured into culture containers and sterilized in an autoclave at 121 ºC and 1.05 atm pressure. The sterilized nutrient medium was then transferred to a sterile cabinet, and the shoot tips were introduced. The shoots were cultured under 16 hours light, 8 hours dark, and at a temperature of 25 ºC.

Development of Encapsulation (Synthetic Seed) Protocol for Shoot Tips and Calculation of Plant Conversion Rates

Shoot tips from two different myrtle genotypes were encapsulated. The shoot tips were first washed in a liquid MS medium without calcium. The structures were transferred to a 3% Na-alginate solution and left in a sterile cabinet at 25°C for 30 minutes. Using a sterile pipette tip cut to 0.5-1 cm, the explants with Na-alginate solution were then transferred into a 100 mM $CaCl_2$ solution (shaking was performed to prevent synthetic seeds from sticking to each other), facilitating the polymerization of the synthetic seeds (20 minutes at 25°C). The polymerized synthetic seeds were then divided into three groups. The first group of synthetic seeds and uncoated shoot tips were transferred to MS (Murashige and Skoog, 1962) media containing 1 mg/L BAP, and the plant conversion rate was determined after 4 weeks. Additionally, the second group of synthetic seeds was stored in sterile jars at +4 °C for 45 days. Afterward, these synthetic seeds were transferred to MS nutrient media containing 1 mg/L BAP to understand the plant conversion rate after 45 days, which was also determined after 4 weeks. Thus, (1) The plant conversion rate of encapsulated synthetic seeds and uncoated shoot tips from myrtle genotypes bearing white and black fruits was calculated as a percentage. (2) The encapsulated synthetic seeds of shoot tips from myrtle genotypes with white and black fruits were stored at +4 °C for 45 days, and the plant conversion rates of these synthetic seeds were calculated as a percentage after 45 days. The potential for short-term conservation of the resulting synthetic seeds was investigated. (3) The third group of synthetic seeds was used in cryopreservation studies to explore the possibilities for long-term conservation of these synthetic seeds.

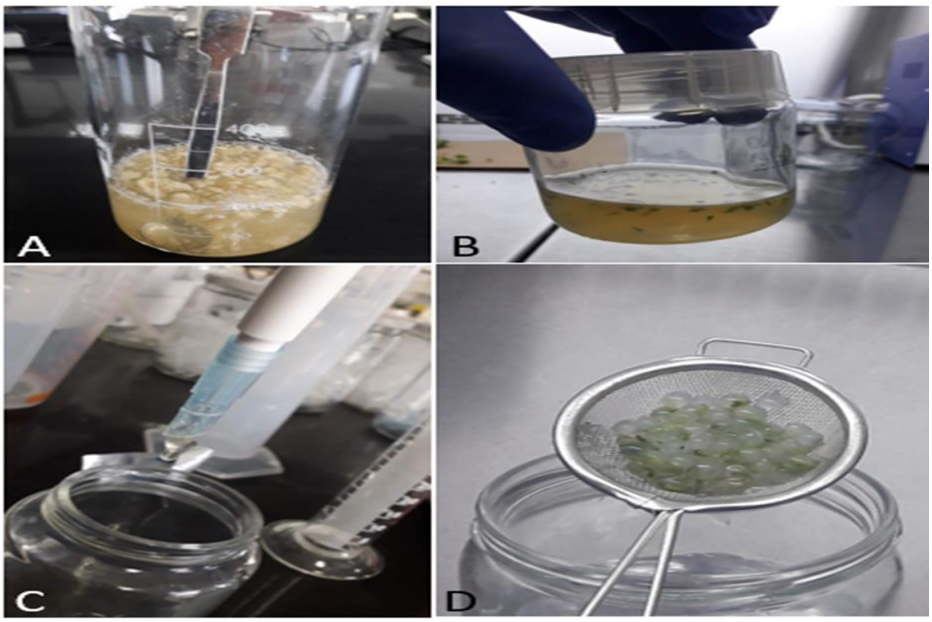

Figure 1. Synthetic Seeds A: Preparation of the Na-Alginate solution. B: Immersion of shoot tips in the sodium alginate solution. C: Coating of shoot tips using a cut pipette tip as a result of polymerization. D: The resulting synthetic seeds.

Cryopreservation Studies

The cryopreservation possibilities for long-term conservation of synthetic seeds obtained by encapsulating shoot tips of *M. communis* genotypes were investigated by freezing them in liquid nitrogen (-196 °C). Before transferring the synthetic seeds into liquid nitrogen, they were subjected to various solutions per the protocol outlined below to prevent cell damage. The thawing processes were carried out after spending at least one hour in liquid nitrogen. Following the thawing, the synthetic seeds were transferred to MS nutrient media containing 1 mg/L BAP, and the plant conversion rate was determined after 4 weeks.

The cryopreservation study followed a detailed protocol for encapsulation of the synthetic seeds obtained from M. communis genotypes' shoot tips. Initially, pre-culture treatments were conducted using MS basal nutrient medium with 2% Mannitol, devoid of hormones, and the explants were kept at +4 °C in darkness for 48 hours. Post-pre-culture, the explants were transferred to cryotubes, and 1.5 ml of loading solution (comprising 2 M glycerol (w/v) and 0.4 M sucrose (w/v)) was added. These were then incubated for 30 minutes at 25 °C. Following this, the Loading solution was removed, and the explants were treated with 1.5 ml of PVS-2 solution (Plant Vitrification Solution-2 containing 30% glycerol (w/v), 15% ethylene glycol (w/v), 15% DMSO (w/v), and 0.4 M sucrose (w/v)). This step involved incubation in PVS-2 for 60 minutes at 0 °C. After this period, the PVS-2 solution was replaced with a fresh 1 ml solution. Subsequently, the cryotubes containing PVS-2 were rapidly immersed in a liquid nitrogen tank at -196 °C and maintained there for at least one hour. After this duration, the cryotubes were removed from the nitrogen tank and quickly thawed in a 38 °C water bath for 2-3 minutes. The PVS-2 solution was then carefully removed from the thawed cryotubes using a sterile syringe, and 1.5 ml of washing solution (Liquid MS basal plus 1.2 M sucrose) was added, followed by an additional incubation period of 20 minutes at 25 °C. Finally, after removing the washing solution, the synthetic seeds from the cryotubes were placed into regeneration media (MS medium containing 1 mg/L BAP) with filter paper. The conversion rate of these synthetic seeds into plants was evaluated after 4 weeks, with the results expressed as a percentage.

Experimental Design, Evaluated Criteria, and Statistical Analyses

The study used three replicates, each consisting of 50 shoot tips (encapsulated, unencapsulated, encapsulated, and stored at +4 °C for 45 days, and encapsulated for cryopreservation, totaling 200 shoot tips for each genotype). The plant conversion rates of the shoot tips were calculated as percentages following all treatments. The statistical significance of the differences between the specified treatments was determined using the T-test. Statistical analyses were conducted using the JMP software package. The treatments compared were as follows: (1) The plant conversion rates (%) for both myrtle genotypes using Na-Alginate encapsulated synthetic seeds compared to unencapsulated shoot tips. (2) The plant conversion rates (%) for both myrtle genotypes using Na-Alginate encapsulated synthetic seeds stored at +4 °C for 45 days compared to unencapsulated shoot tips in the first group. (3) The plant conversion rates (%) for both myrtle genotypes using Na-Alginate encapsulated and cryopreserved synthetic seeds compared to unencapsulated shoot tips in the first group.

Results and Discussion

Plant Conversion Rates of Genotypes

The study used two myrtle genotypes, one with white and the other with black fruits. These genotypes are considered significant plant genetic resources in our country and have many applications. In this context, shoot tips from these myrtle genotypes were encapsulated to produce synthetic seeds. The plant conversion rates (%) of encapsulated synthetic seeds and unencapsulated shoot tips were calculated. Part of the encapsulated shoot tips was stored for short-term preservation at +4 °C for 45 days, while another group of synthetic seeds underwent cryopreservation studies. Consequently, biotechnological methods were explored to devise optimum conservation strategies for our plant genetic resources, which are at risk of loss due to various threats. Initially, the plant conversion rates (%) of both myrtle genotypes using Na-Alginate encapsulated synthetic seeds compared to unencapsulated shoot tips were calculated without any storage or treatment, and the data are presented in Figure 2.

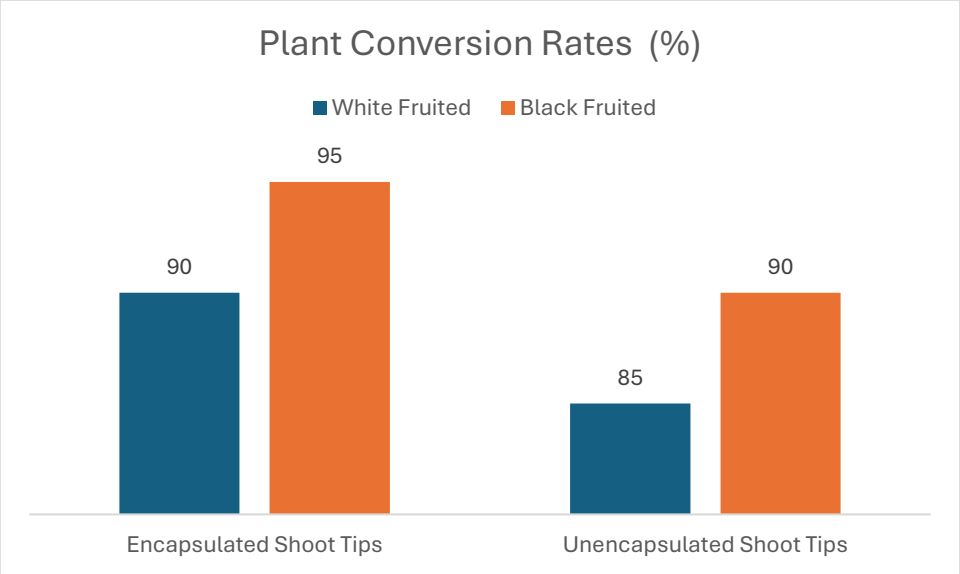

Figure 2. Regeneration rates (%) of coated synthetic seed with Na-Alginate and uncoated shoot tips into plants in both myrtle genotypes.

The plant conversion rates were calculated 4 weeks after the shoot tips of myrtle genotypes, and the resulting encapsulated synthetic seeds were cultured in an MS nutrient medium. For the white-fruited myrtle genotype, the plant conversion rate of unencapsulated shoot tips was determined to be 95%. The plant conversion rate for synthetic seeds was identified as 90%. A T-test conducted between the plant conversion rates of shoot tips and synthetic seeds of the white-fruited myrtle genotype showed no significant statistical difference, demonstrating that the encapsulation process did not adversely affect the plant conversion rate of the synthetic seeds. In the case of the black-fruited myrtle genotype, the plant conversion rate of unencapsulated shoot tips was determined to be 90%, while the conversion rate for synthetic seeds was 85%. A T-test between the plant conversion rates of the black-fruited myrtle genotype's shoot tips and synthetic seeds also showed no significant statistical difference. The plant conversion rate of shoot tips of the white-fruited myrtle genotype was higher than that of the black-fruited genotype.

Additionally, the plant conversion rates (%) of both myrtle genotypes using unencapsulated shoot tips and Na-Alginate encapsulated synthetic seeds stored at +4 °C for 45 days were calculated, and the data are presented in Figure 3.

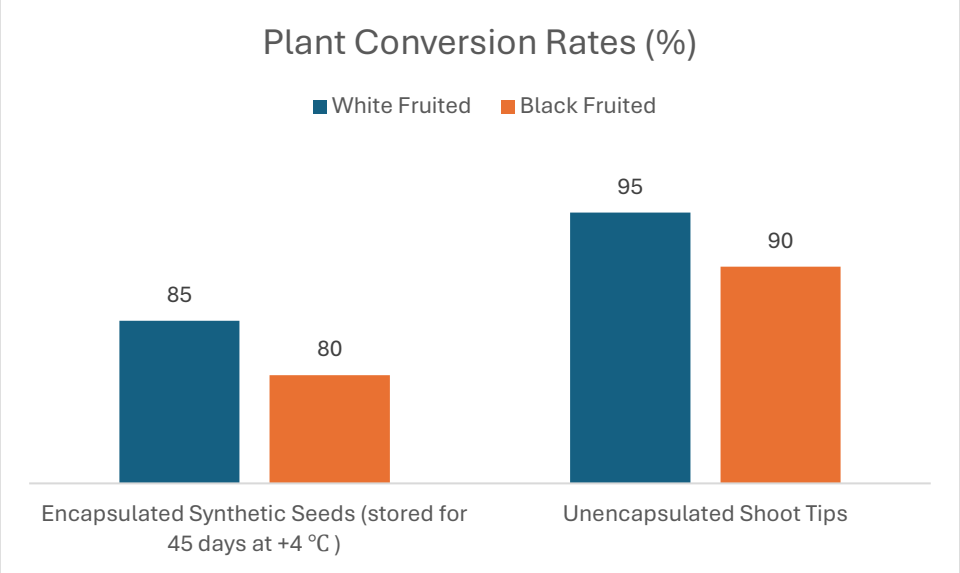

Figure 3. Regeneration rates (%) of Na-Alginate-coated synthetic seeds and uncoated shoot tips of both myrtle genotypes after 45 days at +4 °C.

The synthetic seeds obtained from the encapsulation of shoot tips from myrtle genotypes were stored for 45 days at +4 °C and then cultured in an MS nutrient medium. Four weeks after the initiation of cultivation, the plant conversion rates were calculated as percentages. The data for unencapsulated shoot tips were commonly used for each application. For the white-fruited myrtle genotype, the plant conversion rate of unencapsulated shoot tips was determined to be 95%. The plant conversion rate for synthetic seeds stored at +4 °C for 45 days was 85%. A T-test between the plant conversion rates of shoot tips and stored synthetic seeds for the white-fruited myrtle genotype indicated a higher success rate for unencapsulated shoot tips. However, a conversion rate of 85% after a 45-day storage period is considered a highly successful result, proving that synthetic seeds obtained by encapsulation can be stored at +4 °C for at least 45 days before being converted into plants. In the black-fruited myrtle genotype, the plant conversion rate for unencapsulated shoot tips was 90%, while the conversion rate for synthetic seeds stored for the same duration was 80%. A T-test comparing these rates showed a

higher success rate for unencapsulated shoot tips. Nevertheless, similar to the white-fruited genotype, an 80% conversion rate after a 45-day storage period is highly successful. The plant conversion rate of shoot tips of the white-fruited myrtle genotype was found to be higher than that of the black-fruited genotype. Furthermore, the plant conversion rates (%) for both myrtle genotypes using cryopreserved Na-Alginate encapsulated synthetic seeds compared to unencapsulated and non-cryopreserved shoot tips were calculated, with the data presented in Figure 4.

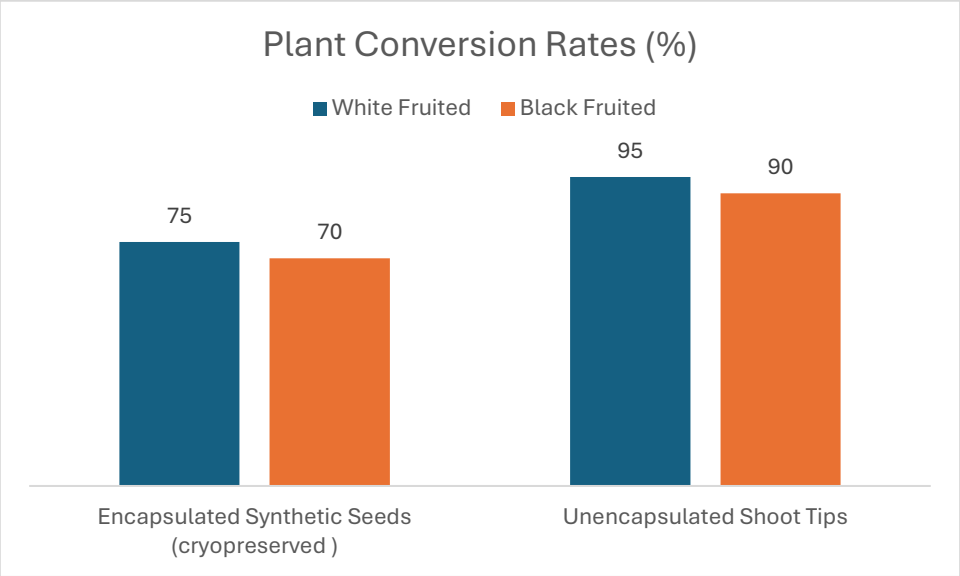

Figure 4. Plant regeneration rates (%) of cryopreserved Na-Alginate-coated synthetic seeds and uncoated and cryopreserved shoot tips in both myrtle genotypes.

In a study on the myrtle genotypes, synthetic seeds derived from encapsulated shoot tips were subjected to cryopreservation and subsequently cultured in the Murashige and Skoog (MS) medium. After four weeks, the conversion rates to plants were calculated as percentages. Data from unencapsulated shoot tips were consistently used across all treatments. In the case of the white-fruited myrtle genotype, the conversion rate of unencapsulated shoot tips to plants was determined to be 95%. For synthetic seeds that underwent cryopreservation, the conversion rate to plants was observed to be 75%. A T-test conducted between the shoot tips of the white-fruited myrtle genotype and the cryopreserved synthetic seeds revealed that the unencapsulated shoot tips had a more successful plant conversion rate. However, the 75% conversion rate observed under preservation conditions at -196 °C was also highly successful. This outcome demonstrates that synthetic seeds obtained through encapsulation can be preserved for desired durations via cryopreservation and converted into plants. In the black-fruited myrtle genotype, the plant conversion rate of unencapsulated shoot tips was identified as 90%. The conversion rate for cryopreserved synthetic seeds in this genotype was 70%. A T-test between the shoot tips and cryopreserved synthetic seeds of the black-fruited myrtle genotype indicated that the unencapsulated shoot tips displayed a more successful conversion rate. Nevertheless, similar to the white-fruited myrtle, the 70% conversion rate post-cryopreservation was deemed a highly successful result, affirming that synthetic seeds can be preserved for extended periods through cryopreservation. Overall, the shoot tips of the white-fruited myrtle genotype showed a higher plant conversion rate compared to the black-fruited myrtle genotype. This study underlines the potential of cryopreservation as a viable method for the long-term conservation of myrtle genotypes and highlights the effectiveness of encapsulation in

enhancing plant regeneration from preserved material.

Figure 5. The appearance of some myrtle plants obtained from synthetic seeds

Plant tissue culture techniques are extensively utilized in biotechnological methods to conserve plant genetic resources. The production of synthetic seeds and their short-term preservation at +4°C or long-term (for as long as desired) storage at -196°C is feasible. Conducting research in this area and optimizing protocols is of utmost importance.

The production of synthetic seeds involves various steps and factors that must be optimized for each plant species. These factors include the selection of explants, the composition of the culture medium, the addition of plant growth regulators, and the encapsulation technique. Different encapsulating materials like sodium alginate or calcium alginate have been used to protect somatic embryos or meristematic tissues. The encapsulated materials are typically treated with a calcium chloride to form a gel-like matrix (Prakash et al., 2018). Research has been conducted on various plant species to develop protocols for synthetic seed production. For instance, studies have been conducted on endangered medicinal plants like *Satureja khuzistanica*, *Swertia chirayita*, and *Mentha arvensis*. These studies have demonstrated successful synthetic seed production and subsequent plant regeneration. In the case of *Satureja khuzistanica*, the encapsulation of microcuttings using sodium alginate resulted in genetically stable plants with high rosmarinic acid content (Asadi et al., 2022). In *Swertia chirayita*, synthetic seed production was achieved through somatic embryogenesis, and the seeds showed high germination rates (Kumar & Chandra, 2013). Similarly, in *Mentha arvensis*, shoot tip, and nodal explants were encapsulated in sodium alginate beads, leading to successful plant regeneration (Islam & Bari, 2014). The success of synthetic seed production depends on various factors such as the choice of explant, composition of the culture medium, encapsulation technique, and the subsequent conversion of synthetic seeds into plants. Optimizing these factors is crucial to achieve high conversion rates and ensure the genetic stability of the regenerated plants (Prakash et al., 2018). Also, storage conditions and post-encapsulation treatments can affect synthetic seeds' viability and conversion efficiency (Taha et al., 2013). In conclusion, synthetic seed production is a valuable technique for the rapid and mass propagation of plant species that do not produce viable seeds or for the conservation and distribution of germplasm. It offers advantages such as uniformity, scalability, and long-term storage potential. The success of synthetic seed production depends on various factors, and their optimization is necessary to achieve high conversion rates and maintain the genetic stability of regenerated plants. Further research and development are needed to enhance and

expand the application of synthetic seed technology across different plant species.

To date, such studies have been carried out on various plant species. Notable examples include citrus (Sekai et al., 1990), apple (Hao et al., 2001), grape (Matsumoto et al., 2003), olive (Bradaï et al., 2021), and avocado (O'Brien et al., 2021). These studies underline the versatility and effectiveness of biotechnological approaches in preserving and maintaining diverse plant genetic materials, showcasing the potential for wide-ranging applications in agriculture and horticulture. Upon reviewing the literature, it appears that no studies specifically address the conservation of genetic resources in the myrtle plant. In a study conducted by Dönmez (2022), synthetic seeds were produced in myrtle plants, but unlike our study, no short or long-term preservation efforts were undertaken. Dönmez focused solely on evaluating the plant conversion rates of the synthetic seeds obtained. In this context, our research has yielded significantly important and practically transferable results, surpassing the scope of Dönmez's study in the literature. This highlights the novelty and relevance of our work in the genetic conservation of myrtle plants, contributing valuable insights into the long-term preservation and effective utilization of synthetic seeds in plant biotechnology.

Synthetic seeds involve encapsulating somatic embryos, shoot tips, or other meristematic tissues in a protective coat, facilitating their transformation into plants under *in vitro* or *in vivo* conditions. This method offers several advantages for rapid and mass propagation, uniformity, and long-term germplasm storage. Synthetic seeds can be used to propagate plant species that do not produce viable seeds, such as seedless grapes and watermelons. Additionally, they are valuable for the conservation and exchange of germplasm and the production of elite plant genotypes (Rihan et al., 2017; Abbas et al., 2022).

Conclusion

The research successfully demonstrates the potential of synthetic seed production and cryopreservation as effective tools for conserving *Myrtus communis* L. genotypes. Our comprehensive approach, involving the encapsulation of shoot tips and their subsequent preservation, offers new insights into long-term germplasm storage. The significant plant conversion rates post-cryopreservation underline the robustness of this method. The study paves the way for advanced conservation strategies, ensuring myrtle plants' safeguarding and genetic stability for future generations. This work contributes to conserving myrtle genotypes and sets a precedent for similar efforts in other plant species.

References

Abbas, M.K., Mahood, H.E., Alhasan, A.S., (2022) Production of synthetic seeds in vegetable crops: A review. In IOP Conference Series: Earth and Environmental Science (Vol. 1060, No. 1, p. 012099). IOP Publishing.

Aleksic, V., Knezevic, P., (2014) Antimicrobial and antioxidative activity of extracts and essential oils of *Myrtus communis* L. *Microbiological Research*, 169(4), 240–254.

Asadi, R., Abdollahi, M.R., Moosavi, S.S., Mirzaie-Asl, A., (2022) Alginate encapsulation of micro-cuttings in endangered *Satureja khuzistanica* species: A promising method for obtaining genetically stable plants with high rosmarinic acid content. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 151(2), pp.307-320.

Bradaï, F., Sánchez-Romero, C. (2021) Effect of cryopreservation on the ex vitro establishment of olive plants regenerated via somatic embryogenesis. *Plants*, 10(2), 396.

Dönmez, D. (2022) Regeneration of plants from alginate-encapsulated shoot tips of myrtle (*Myrtus communis* L.). *Erwerbs-obstbau*, 64(2), 307-314

Engelmann, F. (2004) Plant cryopreservation: progress and prospects. *In Vitro Cellular & Developmental Biology-Plant*, 40, 427-433.

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(4), 599-604.

Grzegorczyk, I., Wysokinska, H. (2011) A protocol for synthetic seeds from *Salvia officinalis* L. shoot tips. *Acta Biologica Cracoviensia. Series Botanica*, 53(1).

Hao, Y. J., Liu, Q. L., Deng, X.X. (2001) Effect of cryopreservation on apple genetic resources at morphological, chromosomal, and molecular levels. *Cryobiology,* 43(1), 46-53.

Islam, M.S. and Bari, M.A. (2012) In vitro regeneration protocol for artificial seed production in an important medicinal plant *Mentha arvensis* L. *Journal of Bio-Science*, 20, pp.99-108.

Kumar, V. and Chandra, S. (2014) High frequency somatic embryogenesis and synthetic seed production of the endangered species *Swertia chirayita*. *Biologia*, 69, pp.186- 192.

Lambardi, M., De Carlo, A. (2003) Application of tissue culture to the germplasm conservation of temperate broad-leaf trees. *Micropropagation of woody trees and fruits*, 815-840.

Matsumoto, T., Sakai, A., (2003) Cryopreservation of axillary shoot tips of in vitro-grown grape (*Vitis*) by a two-step vitrification protocol. *Euphytica*, 131, 299-304.

O'Brien, C., Hiti-Bandaralage, J., Folgado, R., Hayward, A., Lahmeyer, S., Folsom, J., Mitter, N., 2021. Cryopreservation of woody crops: The avocado case. *Plants*, 10(5), 934.

Picci, V., and Atzei, A.D. (1996) Ricerche su Specie di Interesse Medicinale ed Aromatico della Sardegna. *Ricerche Morfo-Sistematiche e Biologiche su Finocchio, Liquirizia*, Mirto,

Prakash, A.V., Nair, D.S., Alex, S., Soni, K.B., Viji, M.M. and Reghunath, B.R. (2018) Calcium alginate encapsulated synthetic seed production in *Plumbago rosea* L. for germplasm exchange and distribution. *Physiology and molecular biology of plants*, 24, pp.963-971.

Rezaee, A., and Kamali, K. (2014) A New Commercial Protocol for Micropropagation of Myrtus tree. *Adv. Biores*, 5(4);73-79.

Rihan, H.Z., Kareem, F., El-Mahrouk, M.E. and Fuller, M.P. (2017) Artificial seeds (principle, aspects and applications). *Agronomy,* 7(4), p.71.

Sakai, A., Kobayashi, S., Oiyama, I. (1990) Cryopreservation of nucellar cells of navel orange (*Citrus sinensis* Osb. var. *brasiliensis* Tanaka) by vitrification. *Plant Cell Reports*, 9(1), 30-33.

Serce, S., Simsek, O., Gunduz, K., Aka-Kacar, Y., Ercisli, S. (2008) Relationships among myrtle accessions from Turkey as revealed by fruit characteristics and RAPD. *Roumanian Biotechnology Letters*, 13(6), 4054-4065.

Şimşek, Ö., Açar, E., Dönmez, D., Şimşek, Ö., Aka Kaçar, Y. (2022) Development of Genic-SSR Markers in Myrtle by RNA-seq. *Erwerbs-Obstbau*, 64(3):475-483. http://doi.org/10.1007/s10341-022-00644-3

Şimşek, Ö., Dönmez, D., Sarıdaş, M.A., Acar, E., Kaçar, Y.A., Kargı, S.P., İzgü, T. (2023) In vitro and ex vitro propagation of Turkish myrtles through conventional and plantform bioreactor systems. *PeerJ*, 11:p.e16061. http://doi.org/10.7717/peerj.16061

Simsek, O., Donmez, D., Saridas, M.A., Paydas-Kargi, S., and Aka-Kacar, Yildiz. (2020) Genetic relationship and polymorphism of Turkish myrtles (*Myrtus communis* L.) as revealed by inter simple sequence repeat (ISSR). *Appl Ecol Environ Res,* 18(1), pp.1141-1149.

Sumbul, S., Aftab Ahmad, M., Asif, M., Akhtar, M. (2011) Myrtus communis Linn. - A review. *Indian Journal of Natural Products and Resources*, *2*(4), 395–402.

Taha, R. M., Mahmad, N., Yaacob, J. S., Abdullah, N., Mohajer, S. (2013) Synthetic Seeds Production and Regeneration of *Oxalis triangularis* for mass propagation and conservation. *IJESD*, 461-464.

Tan, A. (1998) Current status of plant genetic resources conservation in Turkey. In International Symposium on In Situ Conservation of Plant Genetic Diversity, Antalya (Turkey), 4-8 Nov 1996. Central Research Institute for Field Crops.

Towill, LE., Bajaj, Y.P.S. (Eds.). (2002) Cryopreservation of plant germplasm II (Vol. 2). Springer Science & Business Media.