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IN VITRO MICROPROPAGATION OF EUODIA FOR SUSTAINABLE PLANTLET PRODUCTION

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Abstract
This study explores the in vitro micropropagation techniques applied to Euodia species to achieve sustainable and large-scale plantlet production. Given the growing demand for medicinal and ornamental plants, Euodia—a genus known for its pharmacological and ecological significance—has emerged as a key candidate for biotechnological cultivation. The research focuses on optimizing culture conditions, including explant selection, growth media, hormone combinations, and environmental parameters, to ensure high regeneration rates and genetic stability. Results highlight the efficiency of micropropagation in overcoming challenges associated with conventional propagation methods, such as low germination rates and vulnerability to pests. This approach offers a reliable strategy for conservation, commercial cultivation, and the long-term sustainability of Euodia resources.

Keywords: Euodia, in vitro micropropagation, pathogen-free plantlets, sustainable agriculture, environmental restoration, plant biotechnology, regeneration, acclimatization

Introduction

The increasing global demand for medicinal and economically valuable plants has prompted researchers to explore sustainable methods for plant propagation and conservation. Euodia, a genus belonging to the Rutaceae family, is

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recognized for its diverse pharmacological properties, ecological value, and ornamental potential. However, traditional propagation techniques such as seed germination or vegetative cuttings are often inefficient due to low viability, long growth cycles, and susceptibility to environmental stresses.

In vitro micropropagation has emerged as a powerful biotechnological tool to address these limitations by enabling the rapid, large-scale production of genetically uniform and disease-free plantlets. This technique involves cultivating plant tissues under sterile conditions using nutrient-rich media and growth regulators to promote shoot induction, multiplication, and rooting. Applying this method to Euodia not only ensures a consistent supply of planting material but also contributes to biodiversity preservation and sustainable agricultural practices.

This paper aims to investigate and optimize the in vitro conditions for micropropagation of Euodia, offering insights into protocols that can support commercial cultivation, conservation efforts, and the long-term sustainability of this valuable plant resource.

Euodia (Rutaceae), valued for its ecological and phytochemical properties, was investigated for in vitro micropropagation to produce pathogen-free plantlets at the Plant Biotechnology Laboratory, Gulistan State University, Uzbekistan, from 2020 to 2025. Using Murashige and Skoog (MS) medium supplemented with 1.5 mg/L 6-benzylaminopurine (BAP) and 0.5 mg/L indole-3-acetic acid (IAA), apical shoots achieved an 85 ± 3.2% regeneration rate and 70 ± 2.8% callus formation, significantly outperforming lateral shoots (65 ± 2.9%) and leaf segments (40 ± 2.5%) ($n=100$ per explant type; $p<0.001$, ANOVA).

A sterilization protocol involving 70% ethanol and 0.1% mercuric chloride ensured 100% pathogen-free plantlets ($n=200$). Acclimatization in a perlite:peat:sand (1:1:1) substrate resulted in a 90 ± 2.1% success rate (135 of 150 plantlets), with average root length of 5.8 ± 0.7 cm and 6.5 ± 0.5 leaves per plantlet ($p<0.01$). These findings demonstrate the efficacy of in vitro techniques in producing high-quality, disease-resistant Euodia plantlets, addressing

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challenges of pathogen contamination and low propagation efficiency in traditional methods.

The protocol's success supports Euodia's potential for sustainable agriculture, reforestation, and phytochemical applications in Uzbekistan's subtropical and temperate climates. Future research should focus on molecular analyses to confirm genetic stability and field trials to assess long-term adaptability. This study offers a scalable, biotechnological solution for enhancing Euodia cultivation, contributing to global efforts in sustainable plant production and environmental restoration.

In vitro micropropagation of Euodia using Murashige and Skoog (MS) medium supplemented with 1.5 mg/L 6-benzylaminopurine (BAP) and 0.5 mg/L indole-3-acetic acid (IAA) yielded significant outcomes across explant types. Apical shoots achieved the highest regeneration rate of 85 ± 3.2% and callus formation of 70 ± 2.8%, producing an average of 3.2 ± 0.4 shoots per explant ($n=100$; $p<0.001$, ANOVA). Lateral shoots recorded 65 ± 2.9% regeneration and 55 ± 3.1% callus formation with 2.1 ± 0.3 shoots per explant, while leaf segments showed 40 ± 2.5% regeneration and 30 ± 2.3% callus formation with 1.5 ± 0.2 shoots per explant. The sterilization protocol, involving 70% ethanol (30 seconds) and 0.1% mercuric chloride (5 minutes), ensured 100% pathogen-free plantlets ($n=200$), confirmed through microbiological plating. Acclimatization in a perlite:peat:sand (1:1:1) substrate over 4–6 weeks resulted in a 90 ± 2.1% success rate (135 of 150 plantlets), with plantlets developing robust roots (5.8 ± 0.7 cm) and an average of 6.5 ± 0.5 leaves ($p<0.01$; Table 4). Weekly monitoring showed acclimatization success increasing from 20% in week 1 to 90% by week 6, indicating stable adaptation to Uzbekistan's climate (12–15°C, 60–70% humidity).

Conclusion

The in vitro micropropagation of Euodia presents a viable and efficient alternative to conventional propagation methods, particularly in the context of sustainable plantlet production. Through the optimization of culture media, plant growth

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regulators, and sterilization protocols, it is possible to achieve high-frequency shoot induction, effective multiplication rates, and robust root development in a controlled environment. This method significantly reduces the time required for plantlet development and ensures genetic uniformity and disease-free stock, which is critical for both commercial cultivation and conservation strategies.

Furthermore, the successful application of micropropagation techniques to Euodia highlights the broader potential of plant tissue culture in addressing the growing demand for high-value medicinal and horticultural plants. It also provides a foundation for future biotechnological interventions such as secondary metabolite enhancement, germplasm preservation, and genetic improvement. In the face of environmental degradation and overharvesting, in vitro propagation not only offers a scalable solution for sustainable resource utilization but also reinforces the importance of integrating plant biotechnology into ecological and economic frameworks.

Thus, micropropagation of Euodia is not merely a propagation technique but a strategic tool for sustainable development in plant sciences, with far-reaching implications for biodiversity conservation, pharmaceutical industries, and agroforestry systems.

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