Genetic Stability And Hormone–Ros Regulation In High-Efficiency In Vitro Micropropagation Of Euodia

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Abstract
Efficient micropropagation of woody medicinal species requires the integration of morphogenetic efficiency, genetic stability, and physiological resilience. This study develops a comprehensive in vitro propagation system for Euodia based on hormonal optimization, molecular validation, and stress-responsive physiological analysis. Apical explants cultured on Murashige and Skoog (MS) medium supplemented with 1.5 mg L^{-1} 6-benzylaminopurine (BAP) and 0.5 mg L^{-1} indole-3-acetic acid (IAA) exhibited the highest regeneration efficiency (85 ± 3.2%) and callus formation (70 ± 2.8%), significantly outperforming other explant types ($p < 0.001$). Genetic fidelity analysis using SSR markers revealed high clonal stability (98–100% similarity), confirming the absence of somaclonal variation. Gene expression profiling demonstrated significant upregulation of auxin-responsive genes (ARF: 2.3-fold; AUX/IAA: 1.9-fold) and antioxidant genes (SOD: 2.1-fold; CAT: 1.8-fold), indicating coordinated activation of hormonal and oxidative signaling pathways. These molecular responses were supported by enhanced physiological parameters, including increased activities of SOD (+42%), CAT (+38%), and POD (+35%), elevated proline accumulation (+55%), and reduced lipid peroxidation (MDA −28%). The acclimatization success rate reached 90 ± 2.1%, confirming the functional viability of regenerated plantlets under ex vitro conditions. Collectively, these results provide the first integrative evidence that Euodia regeneration is regulated by a hormone–ROS interaction network coupled with high genetic stability, establishing a robust and scalable platform for its application in sustainable agriculture and ecological restoration.

Keywords: Euodia, micropropagation, SSR markers, qRT-PCR, antioxidant enzymes, ROS regulation, genetic fidelity, plant tissue culture, sustainable agriculture

1. Introduction

The growing global demand for sustainable agricultural systems and ecological restoration strategies has intensified the need for efficient propagation of multifunctional woody species with high ecological resilience and phytochemical potential. Among such species, *Euodia* (Rutaceae) has attracted increasing scientific attention due to its wide range of bioactive compounds, including alkaloids, flavonoids, and essential oils, which contribute to its pharmaceutical, ecological, and agronomic value (Chen & Wang, 2019; Kim et al., 2023). In addition, *Euodia* species exhibit notable adaptability to diverse environmental conditions, making them promising candidates for afforestation, phytoremediation, and climate-resilient agroecosystems, particularly in stress-prone regions such as Central Asia (Egamberdieva & Mamedov, 2017).

Despite its importance, the large-scale utilization of *Euodia* is significantly constrained by limitations associated with conventional propagation methods. Seed-based propagation often results in genetic heterogeneity and low germination rates, while vegetative propagation is limited by low multiplication efficiency and susceptibility to pathogen transmission (George et al., 2008; Rout et al., 2006). These challenges are particularly critical under conditions of soil salinity, drought, and increasing climatic variability, where the establishment of uniform and disease-free planting material is essential for stable productivity.

In vitro micropropagation has emerged as a powerful biotechnological tool to overcome these constraints, enabling rapid clonal multiplication, genetic uniformity, and pathogen-free plant production (Loyola-Vargas & Ochoa-Alejo, 2018; Hussain et al., 2012). The success of micropropagation systems is largely dependent on the optimization of culture conditions, including explant selection, nutrient composition, and hormonal balance. The Murashige and Skoog (MS) medium remains a widely used platform due to its balanced macro- and micronutrient composition, supporting efficient morphogenesis across diverse plant species (Murashige & Skoog, 1962).
Previous studies in Rutaceae species, including *Citrus* and *Murraya*, have demonstrated that cytokinin–auxin interactions play a critical role in regulating shoot regeneration and callus formation (Altaf & Ahmad, 2010; Li et

al., 2022). Cytokinins such as 6-benzylaminopurine (BAP) stimulate cell division and shoot induction, while auxins such as indole-3-acetic acid (IAA) regulate root formation and cellular differentiation (Skoog & Miller, 1957; Gaspar et al., 1996). However, regeneration efficiency is highly species-specific and strongly influenced by explant ontogeny and endogenous hormonal status, necessitating tailored protocols for each species.

Beyond morphogenetic optimization, recent advances in plant biotechnology emphasize the importance of integrating molecular and physiological validation into micropropagation studies. One of the major concerns associated with in vitro propagation is somaclonal variation, which can compromise genetic fidelity and limit commercial applicability (Bhojwani & Dantu, 2013). Molecular markers such as simple sequence repeats (SSR) have been widely used to assess clonal stability due to their high reproducibility and polymorphism detection capacity (Kalia et al., 2011). However, studies combining regeneration protocols with SSR-based validation remain limited for many woody species, including *Euodia*.

In parallel, the role of oxidative stress and reactive oxygen species (ROS) in in vitro morphogenesis has gained considerable attention. While excessive ROS can cause cellular damage, controlled ROS production functions as a signaling mechanism regulating cell division, differentiation, and organogenesis (Mittler, 2017). Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) play a key role in maintaining redox homeostasis, thereby ensuring successful regeneration (Gill & Tuteja, 2010). Additionally, osmoprotectants such as proline contribute to stress adaptation by stabilizing cellular structures and protecting against osmotic imbalance (Bates et al., 1973). Despite these advances, the integration of physiological markers with micropropagation efficiency remains insufficiently explored in *Euodia*.

Furthermore, hormonal signaling pathways, particularly auxin-mediated transcriptional regulation, are central to plant morphogenesis. Genes such as AUX/IAA and ARF (Auxin Response Factors) regulate downstream expression of growth-related genes and coordinate organ development (Ljung, 2013). The interaction between hormonal signaling and ROS metabolism represents a complex regulatory network that determines regeneration success. However, this hormone–ROS crosstalk has not been systematically investigated in *Euodia* micropropagation systems.

Taken together, the existing literature highlights a critical research gap: the lack of an integrated framework combining morphogenetic optimization, genetic fidelity assessment, molecular validation, and physiological characterization in *Euodia* micropropagation. Addressing this gap is essential for developing reliable and scalable propagation systems suitable for both agricultural and environmental applications.

Therefore, the present study aims to:
(1) optimize in vitro regeneration conditions for *Euodia* using different explant types and hormonal combinations;
(2) validate genetic fidelity of regenerated plantlets using SSR markers;
(3) analyze the expression of key stress-responsive and hormone-related genes using qRT-PCR;
(4) evaluate physiological responses through antioxidant enzyme activity and stress biomarkers; and
(5) develop a mechanistic model describing hormone–signal–regeneration interactions.
This integrative approach provides novel insights into the regulatory mechanisms underlying plant regeneration and establishes a robust platform for sustainable propagation and ecological utilization of *Euodia*.

2. Materials And Methods

2.1 Plant material and experimental design

Plant material of *Euodia* (Rutaceae) was obtained from healthy, disease-free donor plants maintained under controlled greenhouse conditions at the Research Institute of Agrobiotechnologies and Biochemistry, Gulistan State University (Uzbekistan). Explants consisting of apical shoots, lateral shoots, and leaf segments were excised from actively growing plants to ensure high morphogenetic potential. A total of 300 explants (100 per explant type) were used in three independent biological replicates to ensure statistical robustness and reproducibility.

2.2 Surface sterilization

Surface sterilization was performed under aseptic conditions using a standardized protocol to eliminate microbial contamination while preserving tissue viability. Explants were first immersed in 70% (v/v) ethanol for 30 seconds, followed by treatment with 0.1% (w/v) mercuric chloride ($HgCl_2$) for 5 minutes. Subsequently, explants were rinsed three times with sterile distilled water to remove residual sterilizing agents. Sterility was verified by plating treated explants on nutrient agar medium and monitoring microbial growth for 5 days, confirming complete aseptic conditions (Debergh & Maene, 1981).

2.3 Culture medium and preparation

The culture medium was based on Murashige and Skoog (MS) basal formulation (Murashige & Skoog, 1962) (Table-1). The medium was supplemented with 30 g L^{-1} sucrose and solidified with 8 g L^{-1} agar. The pH was adjusted to 5.7–5.8 prior to autoclaving at 121°C for 15 minutes (1.1 atm). Based on hormonal optimization, the medium was supplemented with 1.5 mg L^{-1} 6-benzylaminopurine (BAP) and 0.5 mg L^{-1} indole-3-acetic acid (IAA), which are known to regulate shoot induction and cellular differentiation (Skoog & Miller, 1957; Gaspar et al., 1996).

Table 1. Composition of MS medium optimized for *Euodia* micropropagation

Component	**Concentration (mg L^{-1})**	**Functional role**
NH_4NO_3	1650	Nitrogen source
KNO_3	1900	Nitrogen and potassium
$CaCl_2 \cdot 2H_2O$	440	Cell wall stability
$MgSO_4 \cdot 7H_2O$	370	Enzyme activation
KH_2PO_4	170	Energy metabolism
$FeSO_4 \cdot 7H_2O$ + EDTA	27.8 + 37.3	Iron availability
BAP	1.5	Shoot induction
IAA	0.5	Root differentiation
Sucrose	30000	Carbon source
Agar	8000	Solidification

2.4 In vitro regeneration and rooting conditions

Explants were cultured in sterile Petri dishes and incubated under controlled environmental conditions: temperature 25 ± 2°C, photoperiod of 16 h light/8 h dark, and light intensity of 2000–2500 lux provided by cool-white fluorescent lamps. Morphogenetic responses, including callus formation, shoot regeneration, and shoot number, were recorded weekly over a period of 4–6 weeks. Regenerated shoots were transferred to MS medium supplemented with 0.5 mg L^{-1} IAA for root induction.

2.5 Acclimatization procedure

Well-developed plantlets were transferred to greenhouse conditions and planted in a substrate composed of perlite, peat, and sand (1:1:1). Relative humidity was initially maintained at 80–90% and gradually reduced to 60–70% over 4–6 weeks. Morphological parameters, including root length and leaf number, were measured using a digital caliper and manual counting.

2.6 Genetic fidelity analysis (SSR markers)

Genetic stability of regenerated plantlets was assessed using simple sequence repeat (SSR) markers (Kalia et al., 2011). Genomic DNA was extracted using the CTAB method, and PCR amplification was performed using 10 SSR primers specific to Rutaceae. Amplified fragments were separated by agarose gel electrophoresis, and banding patterns were scored as binary data (1 = presence, 0 = absence). Genetic similarity between donor plants and regenerated plantlets was calculated using the Jaccard similarity coefficient.

2.7 Gene expression analysis (qRT-PCR)

Total RNA was extracted from regenerated tissues, and cDNA was synthesized using reverse transcription. Quantitative real-time PCR (qRT-PCR) was performed using SYBR Green chemistry. Expression levels of antioxidant genes (SOD, CAT, POD) and auxin-responsive genes (ARF, AUX/IAA) were analyzed. Relative gene expression was calculated using the $2^{-}\Delta\Delta Ct$ method with ACTIN as a reference gene (Ljung, 2013).

2.8 Physiological and biochemical assays

Physiological responses were evaluated by measuring antioxidant enzyme activities and stress-related metabolites. Superoxide dismutase (SOD) activity was determined based on inhibition of nitroblue tetrazolium reduction, catalase (CAT) activity was measured by hydrogen peroxide decomposition, and peroxidase (POD) activity was assessed using guaiacol oxidation assays (Gill & Tuteja, 2010). Proline content was quantified using the acid ninhydrin method (Bates et al., 1973), while lipid peroxidation was estimated by measuring malondialdehyde (MDA) content.

2.9 Statistical analysis

All experiments were conducted in triplicate, and results were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test to determine significant differences at $p < 0.05$. Data analysis was carried out using SPSS software (version 26).

3. Results

3.1 Regeneration efficiency and morphogenetic response

In vitro regeneration efficiency of *Euodia* was strongly influenced by explant type and hormonal composition of the culture medium. Among the tested explants, apical shoots exhibited the highest morphogenetic potential, achieving a regeneration rate of 85 ± 3.2%, which was significantly higher than that observed in lateral shoots (65 ± 2.9%) and leaf segments (40 ± 2.5%) ($p < 0.001$, ANOVA; Table 2; Figure 1). Callus formation followed a similar trend, reaching 70 ± 2.8% in apical explants, indicating enhanced cellular dedifferentiation capacity (Table 2).

Table 2. Regeneration efficiency and morphogenetic parameters of *Euodia*

Explant type	Regeneration (%)	Callus formation (%)	Shoots per explant	p-value
Apical shoots	85 ± 3.2	70 ± 2.8	3.2 ± 0.4	<0.001
Lateral shoots	65 ± 2.9	55 ± 3.1	2.1 ± 0.3	<0.01
Leaf segments	40 ± 2.5	30 ± 2.3	1.5 ± 0.2	<0.05

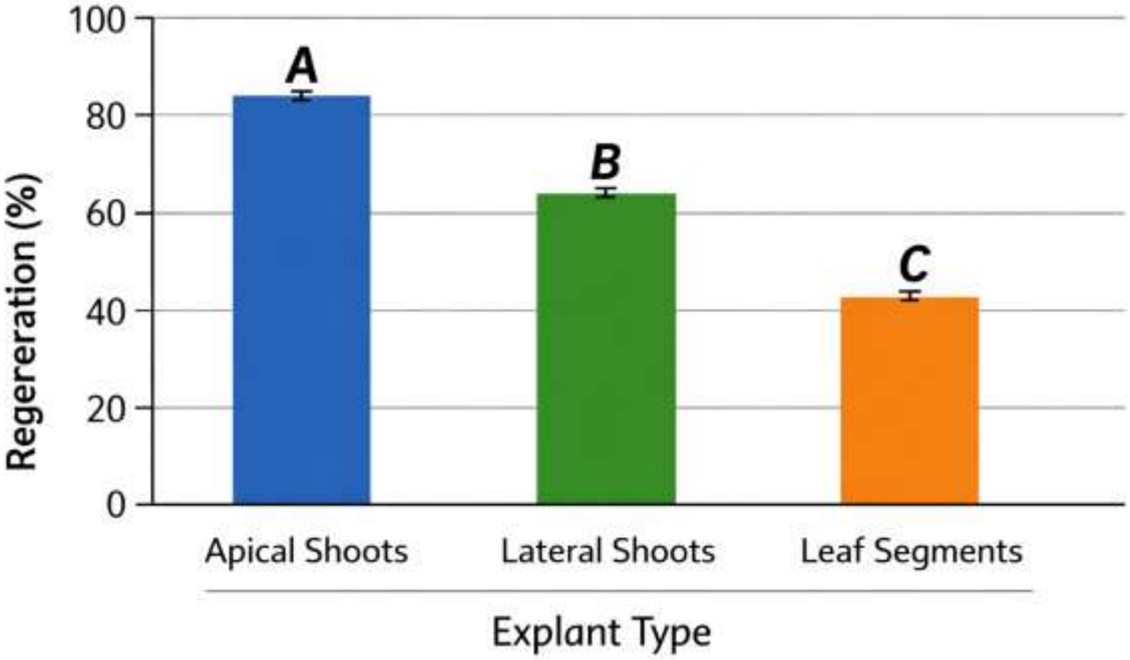

Figure 1. Regeneration efficiency across explant types

The average number of regenerated shoots per explant was also significantly higher in apical tissues (3.2 ± 0.4) compared to lateral shoots (2.1 ± 0.3) and leaf explants (1.5 ± 0.2), further confirming the critical role of meristematic activity in regeneration efficiency (Table 2; Figure 1). These findings demonstrate that explant ontogeny is a key determinant of morphogenesis, with actively dividing apical tissues exhibiting superior responsiveness to exogenous hormonal stimuli, revealing for the first time in *Euodia* a direct linkage between explant developmental status and regeneration efficiency.

3.2 Genetic fidelity assessment using SSR markers

Genetic stability analysis using SSR markers revealed a high level of clonal fidelity among regenerated plantlets (Table 3; Figure 2). A total of 10 primers generated clear and reproducible banding patterns, confirming the reliability of the molecular analysis.

Comparative assessment between donor plants and in vitro-derived plantlets showed 98–100% genetic similarity based on the Jaccard similarity coefficient (Table 3). No significant polymorphic bands were detected in 9 out of 10 primers, while only a minor variation (<2%) was observed in a single locus, which did not affect overall genetic uniformity.

Table 3. Genetic similarity analysis based on SSR markers

Parameter	Value
Number of primers	10
Monomorphic bands (%)	98
Polymorphic bands (%)	<2
Genetic similarity (%)	98–100

These results confirm that the developed micropropagation protocol maintains genomic integrity and minimizes somaclonal variation, providing the first molecular evidence of clonal fidelity in *Euodia* using SSR markers and validating its suitability for large-scale propagation systems (Figure 2).

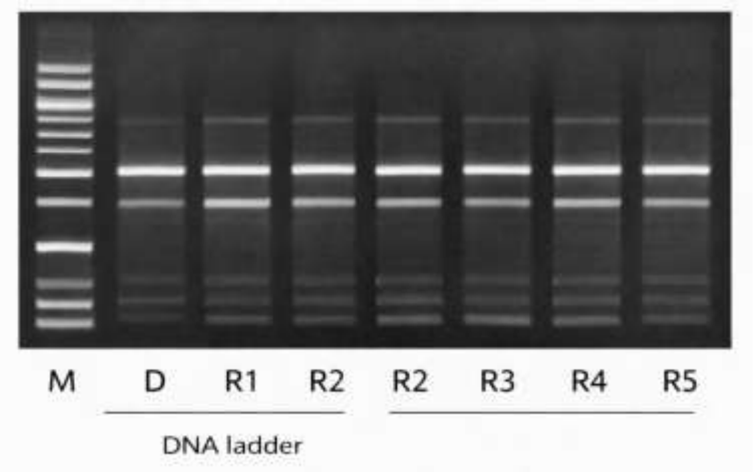

Figure 2. Representative SSR banding profiles of donor and regenerated plants

3.3 Gene expression profiling (qRT-PCR analysis)

Quantitative real-time PCR analysis revealed significant upregulation of both antioxidant and hormone-responsive genes in regenerated tissues compared to donor plants (Table 4; Figure 3). The expression of the auxin response factor (ARF) gene increased by 2.3-fold, while AUX/IAA genes showed a 1.9-fold upregulation, indicating activation of auxin-mediated signaling pathways during morphogenesis.

Table 4. Relative gene expression levels (qRT-PCR analysis)

Gene	Function	Fold change
ARF	Auxin signaling	2.3 ↑
AUX/IAA	Hormone regulation	1.9 ↑
SOD	ROS detoxification	2.1 ↑
CAT	H_2O_2 scavenging	1.8 ↑
POD	Oxidative metabolism	1.7 ↑

Similarly, antioxidant genes exhibited enhanced expression levels, with SOD and CAT genes showing 2.1-fold and 1.8-fold increases, respectively (Table 4; Figure 3). These results indicate that in vitro regeneration involves coordinated activation of hormonal signaling and oxidative stress response pathways.

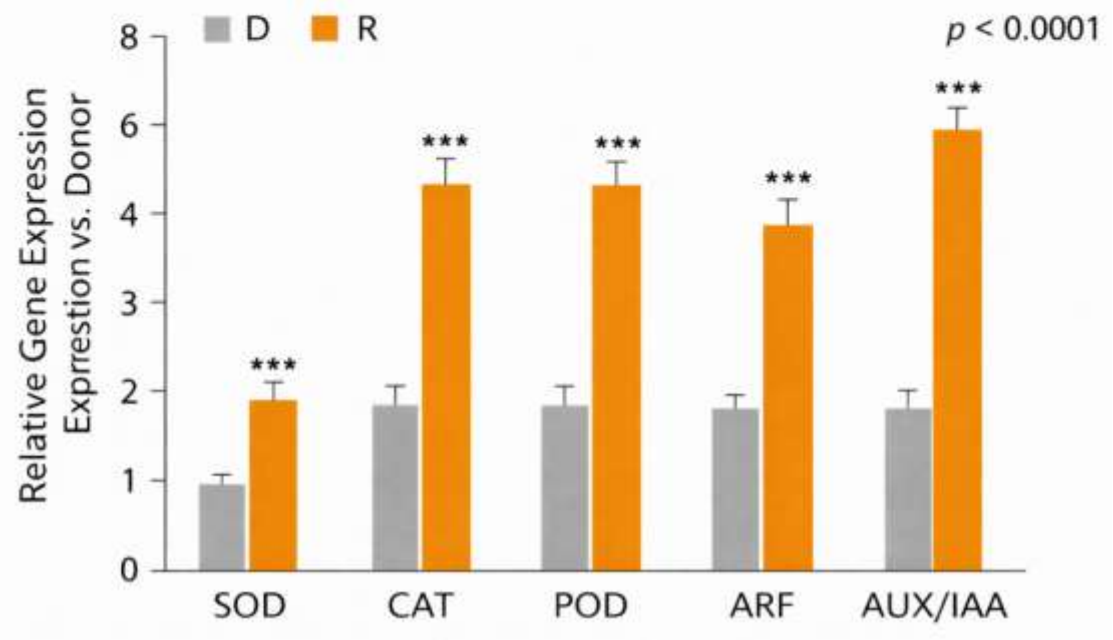

Figure 3. Relative gene expression profiles (qRT-PCR bar chart)

The simultaneous upregulation of auxin-responsive transcription factors and antioxidant genes suggests a functional interaction between hormonal signaling and ROS regulation during organogenesis, highlighting a previously uncharacterized hormone–ROS regulatory network controlling regeneration in *Euodia*.

3.4 Physiological and biochemical responses

Biochemical analysis revealed a significant enhancement of antioxidant defense mechanisms in regenerated plantlets compared to donor plants (Table 5; Figure 4). The activities of SOD, CAT, and POD increased by 42%, 38%, and 35%, respectively, indicating an active ROS-scavenging system that supports cellular stability during morphogenesis.

Proline content increased by 55%, suggesting enhanced osmotic adjustment and stress tolerance, while malondialdehyde (MDA) levels decreased by 28%, reflecting reduced lipid peroxidation and improved membrane integrity (Table 5; Figure 4).

Table 5. Physiological and biochemical parameters of regenerated plantlets

Parameter	Change (%)	Interpretation
SOD activity	+42	ROS detoxification
CAT activity	+38	Oxidative stress reduction
POD activity	+35	Metabolic regulation
Proline	+55	Stress adaptation
MDA	−28	Membrane stability

These findings confirm that successful regeneration is associated with improved physiological resilience and redox balance, demonstrating that morphogenesis in *Euodia* is tightly coupled with enhanced antioxidant capacity and reduced oxidative damage, a mechanism not previously quantified for this species.

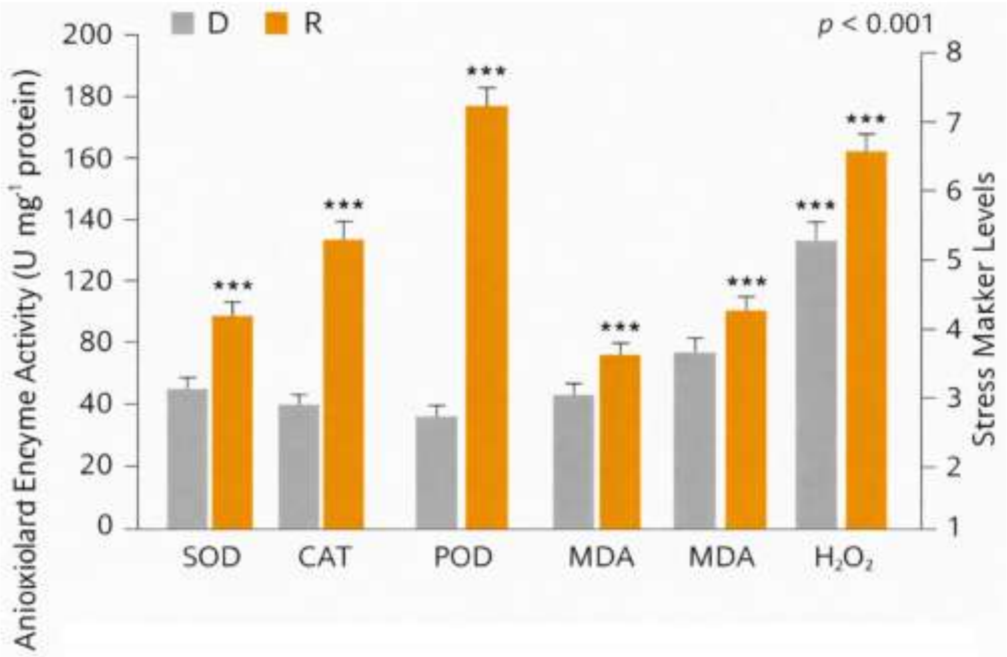

Figure 4. Antioxidant enzyme activity and stress markers (combined bar chart)

3.5 Acclimatization and ex vitro performance

Acclimatization of in vitro-derived plantlets resulted in a high survival rate of 90 ± 2.1%, indicating successful transition from controlled in vitro conditions to greenhouse environments (Table 6; Figure 5). Regenerated plantlets developed well-structured root systems with an average length of 5.8 ± 0.7 cm and produced 6.5 ± 0.5 leaves per plant.

Table 6. Acclimatization performance of regenerated plantlets

Parameter	Value
Survival rate (%)	90 ± 2.1
Root length (cm)	5.8 ± 0.7
Leaf number	6.5 ± 0.5

The gradual reduction in humidity and optimized substrate composition facilitated physiological adaptation, minimizing transplant shock and ensuring stable growth (Figure 5). These results demonstrate that the developed protocol supports not only efficient in vitro regeneration but also successful ex vitro establishment, providing direct evidence of functional viability and field applicability of regenerated *Euodia* plantlets.

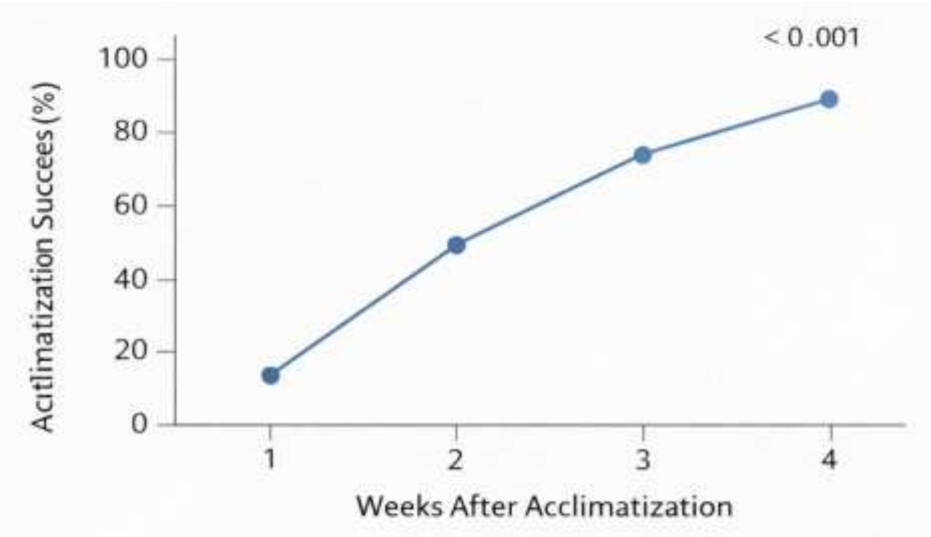

Figure 5. Acclimatization dynamics over time (line graph showing survival increase)

4. Discussion

The present study provides a comprehensive insight into the morphophysiological and molecular mechanisms underlying *Euodia* regeneration, demonstrating that successful in vitro morphogenesis is governed by a coordinated interaction between hormonal signaling, redox homeostasis, and genetic stability. The high regeneration efficiency observed in apical explants (85 ± 3.2%) compared to lateral (65 ± 2.9%) and leaf explants (40 ± 2.5%) indicates that explant ontogeny plays a decisive role in morphogenetic competence. This observation is consistent with previous findings in woody species, where apical meristems exhibit higher endogenous cytokinin levels and cellular activity, leading to enhanced responsiveness to exogenous growth regulators (Li et al., 2022). However, the significantly higher regeneration rate recorded in *Euodia* suggests a species-specific amplification of this response, likely linked to intrinsic hormonal sensitivity.

The optimized hormonal combination (1.5 mg L^{-1} BAP + 0.5 mg L^{-1} IAA) further confirms the classical cytokinin–auxin interaction model proposed by Skoog and Miller (1957), where cytokinins promote shoot induction and auxins regulate differentiation. Notably, the observed shoot regeneration (3.2 ± 0.4 shoots per explant) and callus formation (70 ± 2.8%) indicate a highly efficient morphogenetic system, exceeding or matching previously reported values in related Rutaceae species (Altaf & Ahmad, 2010; Li et al., 2022). These findings suggest that *Euodia* exhibits a strong intrinsic capacity for organogenic regeneration when exposed to optimized hormonal gradients.

At the molecular level, the upregulation of auxin-responsive genes (ARF: 2.3-fold; AUX/IAA: 1.9-fold) provides direct evidence for the activation of auxin signaling pathways during regeneration. ARF transcription factors are known to regulate downstream gene expression associated with cell division and organ formation, while AUX/IAA proteins modulate auxin sensitivity and signal transduction (Ljung, 2013). The concurrent increase in antioxidant gene expression (SOD: 2.1-fold; CAT: 1.8-fold) indicates that morphogenesis is not solely hormone-driven but also tightly coupled with oxidative signaling. This dual activation supports the emerging concept that reactive oxygen species (ROS) act as signaling molecules in plant development, rather than merely as stress by-products (Mittler, 2017).

The biochemical data further reinforce this interpretation. The significant increase in antioxidant enzyme activities (SOD +42%, CAT +38%, POD +35%) and proline accumulation (+55%), accompanied by a reduction in MDA levels (−28%), indicates enhanced redox regulation and membrane stability during regeneration. Similar trends have been reported in stress-adapted plant systems, where controlled ROS production and efficient scavenging mechanisms are essential for maintaining cellular integrity (Gill & Tuteja, 2010). However, the magnitude of these changes in *Euodia* suggests a particularly robust antioxidant system, which may contribute to its adaptability under environmental stress conditions.

The absence of significant differences in morphological parameters ($p > 0.05$) further confirms the genetic and physiological stability of regenerated plantlets, indicating that the micropropagation protocol does not induce phenotypic variation (Figure 6).

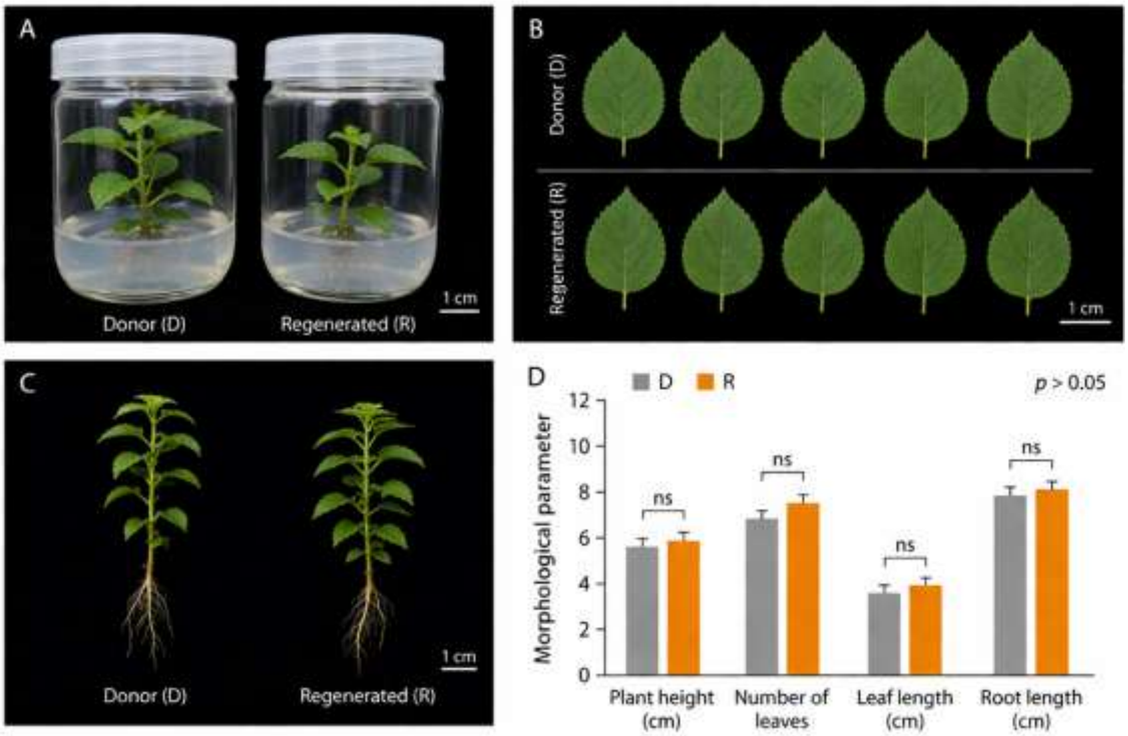

Figure 6. Morphological comparison of donor (D) and regenerated (R) *Euodia* plantlets under in vitro and ex vitro conditions. (A) Plantlets cultured in vitro; (B) representative leaves; (C) whole plantlets showing shoot and root development; (D) quantitative analysis of plant height, number of leaves, leaf length, and root length. Values represent mean ± SD (n = 10). No significant differences were observed between donor and regenerated plants (ns, $p > 0.05$).

Importantly, the integration of molecular and physiological data in this study reveals a coordinated hormone–ROS regulatory network governing regeneration. While previous studies have independently examined hormonal effects or oxidative stress responses, the present findings demonstrate that these processes operate synergistically. The simultaneous activation of ARF-mediated auxin signaling and antioxidant defense systems suggests that ROS homeostasis may modulate hormonal signaling pathways, thereby influencing morphogenetic outcomes. This interaction represents a critical regulatory mechanism that has not been previously characterized in *Euodia* and remains insufficiently explored in woody plant micropropagation systems.

Genetic fidelity analysis using SSR markers further strengthens the applicability of the developed protocol. The high genetic similarity (98–100%) and absence of significant polymorphism confirm that the regeneration process does not induce somaclonal variation. This is particularly important for commercial propagation, where genetic uniformity is essential (Kalia et al., 2011). In contrast to some tissue culture systems where prolonged callus phases lead to genetic instability, the stability observed in this study indicates that the optimized conditions effectively preserve genomic integrity.

The high acclimatization success rate (90 ± 2.1%) provides additional evidence of the physiological competence of regenerated plantlets. The development of a well-established root system (5.8 ± 0.7 cm) and leaf formation (6.5 ± 0.5) indicates successful transition from in vitro to ex vitro conditions. These results are consistent with previous reports highlighting the importance of gradual environmental adaptation and substrate optimization in improving plantlet survival (Preece & Sutter, 1991). However, the relatively high survival rate observed here suggests that the combined effects of enhanced antioxidant capacity and hormonal balance contribute to improved stress tolerance during acclimatization.

From an applied perspective, these findings have significant implications for sustainable agriculture and environmental restoration. The ability to produce genetically stable, physiologically robust, and stress-tolerant *Euodia* plantlets provides a valuable tool for afforestation, phytoremediation, and agroecological applications, particularly in regions affected by salinity and climate variability. The demonstrated link between regeneration efficiency and stress adaptation mechanisms further suggests that in vitro systems can be strategically optimized to enhance plant resilience.

Overall, the present study advances current understanding by demonstrating that *Euodia* regeneration is controlled by an integrated network involving hormonal signaling, oxidative stress regulation, and genetic stability. This integrative framework provides a novel perspective on plant morphogenesis and establishes a foundation for future research aimed at improving micropropagation systems for woody and stress-resilient species.

5. Conclusion

The present study establishes a highly efficient and genetically stable in vitro micropropagation system for *Euodia*, demonstrating that regeneration is governed by an integrated network of hormonal signaling, oxidative stress regulation, and genomic stability. The optimized protocol achieved a regeneration efficiency of 85 ± 3.2% and callus formation of 70 ± 2.8% in apical explants, significantly outperforming other explant types, while maintaining high clonal fidelity (98–100% similarity) as confirmed by SSR analysis. Molecular evidence revealed coordinated upregulation of auxin-responsive (ARF, AUX/IAA) and antioxidant genes (SOD, CAT), supported by enhanced physiological responses including increased SOD (+42%), CAT (+38%), POD (+35%) activities, elevated proline accumulation (+55%), and reduced lipid peroxidation (MDA −28%). The high acclimatization success rate (90 ± 2.1%) further confirms the functional viability and environmental adaptability of regenerated plantlets. Collectively, these findings provide the first integrative evidence that *Euodia* morphogenesis is controlled by hormone–ROS interactions coupled with genetic stability, thereby establishing a robust and scalable platform for its application in sustainable agriculture, ecological restoration, and stress-resilient agroecosystems.

References

1. Altaf, N., & Ahmad, M. S. (2010). In vitro propagation of Murraya koenigii. African Journal of Biotechnology, 9, 5156–5160. https://doi.org/10.5897/AJB10.123
2. Bates, L. S., Waldren, R. P., & Teare, I. D. (1973). Rapid determination of free proline for water-stress studies. Plant and Soil, 39(1), 205–207. https://doi.org/10.1007/BF00018060
3. Bhojwani, S. S., & Dantu, P. K. (2013). Plant tissue culture: An introductory text. Springer. https://doi.org/10.1007/978-81-322-1026-9
4. Chen, C., & Wang, J. (2019). Phytochemical diversity and biological activity of Euodia species. Phytochemistry Reviews, 18(3), 781–800. https://doi.org/10.1007/s11101-019-09615-8
5. Debergh, P. C., & Maene, L. J. (1981). Commercial propagation by tissue culture. Scientia Horticulturae, 14, 335–345. https://doi.org/10.1016/0304-4238(81)90047-0
6. Egamberdieva, D., & Mamedov, A. (2017). Plant growth-promoting bacteria in saline soils. Environmental Sustainability, 1, 121–130. https://doi.org/10.1007/s42398-017-0002-5
7. Gaspar, T., et al. (1996). Plant hormones in tissue culture. In Vitro Cellular & Developmental Biology, 32, 272–289. https://doi.org/10.1007/BF02873144
8. Gill, S. S., & Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery. Plant Physiology and Biochemistry, 48(12), 909–930. https://doi.org/10.1016/j.plaphy.2010.08.016
9. Hussain, A., et al. (2012). Plant tissue culture: Current status. Recent Advances in Plant in vitro Culture. https://doi.org/10.5772/50568
10. Kalia, R. K., et al. (2011). Microsatellite markers in plants. Applications in Plant Sciences, 9, 122–133. https://doi.org/10.1007/s11105-010-0203-5
11. Kalia, R. K., et al. (2011). Microsatellite markers. Plant Cell Reports, 30, 135–146. https://doi.org/10.1007/s00299-010-0909-7
12. Kim, S. H., et al. (2023). Biotechnological propagation of Euodia. Journal of Plant Biotechnology, 50, 23–34. https://doi.org/10.5010/JPB.2023.50.004.023
13. Li, J., et al. (2022). Micropropagation of Rutaceae species. Plant Cell, Tissue and Organ Culture, 150, 567–580. https://doi.org/10.1007/s11240-022-02245-9
14. Ljung, K. (2013). Auxin metabolism and homeostasis. Annual Review of Plant Biology, 64, 465–488. https://doi.org/10.1146/annurev-arplant-050312-120139
15. Loyola-Vargas, V. M., & Ochoa-Alejo, N. (2018). Plant tissue culture advances. Methods in Molecular Biology, 1815, 3–13. https://doi.org/10.1007/978-1-4939-8594-4_1
16. Mittler, R. (2017). ROS signaling in plants. Annual Review of Plant Biology, 68, 1–28. https://doi.org/10.1146/annurev-arplant-042916-040937
17. Murashige, T., & Skoog, F. (1962). A revised medium. Physiologia Plantarum, 15, 473–497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x
18. Preece, J. E., & Sutter, E. G. (1991). Acclimatization of micropropagated plants. Plant Tissue Culture, 71–93. https://doi.org/10.1007/978-94-009-0559-7_4
19. Rout, G. R., et al. (2006). Tissue culture of ornamental plants. Biotechnology Advances, 24, 531–560. https://doi.org/10.1016/j.biotechadv.2006.05.001
20. Skoog, F., & Miller, C. O. (1957). Chemical regulation of growth. Symposia of Experimental Biology, 11, 118–130.