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Direct organogenesis and molecular characterization of *Saintpaulia ionantha* regenerants via SCoT markers

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Abstract
The African violet (*Saintpaulia ionantha* H. Wendl.) is a valuable ornamental species widely used as a model plant in biotechnology due to its high regenerative capacity and adaptability to in vitro culture conditions. This study aimed to evaluate the regeneration and multiplication potential of *S. ionantha* via direct organogenesis and to assess the genetic stability of regenerated plantlets using SCoT (Start Codon Targeted) molecular markers. Micropropagation was performed on Murashige–Skoog (MS) medium supplemented with three hormonal combinations: V1 – 2 mg/l BA, V2 – 2 mg/l BA + 0.5 mg/l NAA, and V3 – 2 mg/l BA + 1 mg/l NAA. Leaf explants were cultured under controlled environmental conditions, and regenerated fragments from ten plantlets were subjected to genomic DNA extraction and SCoT marker analysis (primers T4, T5, T7). The results demonstrated a high efficiency of direct organogenesis, with variant V3 yielding the highest number of regenerants and greatest shoot height. Molecular analysis revealed no detectable polymorphisms among regenerants, indicating strong genetic stability and minimal somaclonal variation. Overall, the study confirms the suitability of direct organogenesis for efficient micropropagation of *S. ionantha* and highlights SCoT markers as reliable tools for evaluating clonal fidelity.

Keywords: tissue culture, plant regeneration, genetic stability, molecular markers; SCoT

Introduction

Saintpaulia ionantha (H. Wendl.), commonly known as the African violet, is one of the most popular ornamental indoor plants worldwide due to its attractive floral diversity, continuous blooming, and adaptability to indoor cultivation conditions [4]. Beyond its horticultural relevance, the species has gained considerable importance as an experimental model in plant biotechnology, particularly because of its strong regenerative capacity and predictable morphogenetic response to plant growth regulators under in vitro conditions [5] [1].

In vitro culture techniques play a central role in the propagation and improvement of ornamental species such as *S. ionantha*, enabling rapid large-scale multiplication, production of pathogen-free plants, and long-term preservation of valuable germplasm. Numerous studies have demonstrated that regeneration in this species can be efficiently induced on Murashige and Skoog (MS) medium supplemented with appropriate combinations of cytokinins and auxins. Previous research has shown that explant type and growth regulator balance—particularly combinations involving BA, IAA, or NAA—strongly influence organogenesis and shoot induction [10]; [13]; [8]. Similar findings were reported by Stănică et al. [17], who developed an efficient organogenesis-based regeneration protocol for multiple *S. ionantha* cultivars using BA and IAA.

Despite consistent progress in micropropagation protocols, plant tissue culture procedures may induce somaclonal variation—genetic or epigenetic changes that arise during in vitro culture. Such variation may compromise the uniformity, stability, and horticultural value of regenerated plant material [2]. For this reason, assessing genetic fidelity is essential when developing micropropagation systems, especially for ornamental species where phenotypic uniformity is critical.

Molecular markers represent powerful tools for evaluating clonal stability, as they allow precise detection of DNA polymorphisms and identification of subtle genetic changes [13]; [12]; [10]; [7]. Among these, Start Codon Targeted (SCoT) markers have recently emerged as a robust, gene-targeted approach based on

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conserved regions flanking the ATG start codon [3]. Their high reproducibility, low cost, and independence from prior sequence information make them particularly suitable for assessing genetic stability in micropropagated plants [6].

Although numerous studies have addressed the in vitro regeneration of *S. ionantha*, limited research has combined direct organogenesis systems with SCoT-based molecular analysis. Furthermore, few studies have examined how different hormonal compositions influence not only regeneration efficiency but also the genetic stability of regenerated plantlets. This highlights the need for an integrated evaluation of morphogenetic response and molecular fidelity.

The present study aims to:

1. investigate the regeneration and multiplication potential of *Saintpaulia ionantha* through direct organogenesis using leaf explants cultured on different hormonal variants;
2. assess the genetic variability and clonal stability of regenerated plantlets using SCoT molecular markers; and
3. correlate regeneration performance with detected genetic variation in order to identify optimal in vitro conditions ensuring both high efficiency and genetic fidelity.

Material and Method

1. In vitro culture

1.1. Plant material and culture initiation

Healthy, vigorously growing leaves of *Saintpaulia ionantha* H. Wendl. were used as initial explants. Prior to culture initiation, explants were surface-sterilized using the following protocol:

1. Washing under running tap water with mild detergent
2. Rinsing with distilled water
3. Immersion in 70% (v/v) ethanol for 20 seconds
4. Disinfection in 0.1% (w/v) $HgCl_2$ for 2 minutes
5. Five to six consecutive rinses with sterile distilled water, each lasting 2–3 minutes

Sterilized leaf fragments were transferred aseptically onto Murashige and Skoog (MS) medium [9] supplemented with specific combinations of plant growth regulators. Three hormonal variants were tested (table 1), each inoculated with three leaf segments per vessel.

Table 1. In vitro culture conditions for organogenesis induction

Variant	Growth regulators	Environmental conditions
V1	2 mg/l BA	24°C, 16 h light / 8 h dark photoperiod, 50–70% relative humidity
V2	2 mg/l BA + 0.5 mg/l NAA	
V3	2 mg/l BA + 1 mg/l NAA	

Cultures were maintained at **25 ± 1°C** in a growth chamber under a controlled environment. Relative humidity was kept between **50–70%** to prevent desiccation of the medium and explants. Excessive humidity was avoided to prevent fungal and bacterial contamination, which would compromise the aseptic conditions essential for *in vitro* growth.

1.2. Shoot regeneration via direct organogenesis

Regeneration occurred directly from leaf explants, without an intervening callus phase. Explants were maintained on their respective media until the appearance of meristematic primordia and adventitious shoots. After eight weeks, shoot height and the number of regenerants per explant were recorded.

1.3. In vitro rooting

Well-developed shoots were transferred to hormone-free MS medium to induce rooting. Cultures were incubated at 24°C under a 16 h photoperiod. Root formation occurred within 2–3 weeks, after which plantlets with well-developed root systems were selected for acclimatization.

1.4. Acclimatization

Rooted plantlets were carefully removed from the medium, rinsed with sterile water to eliminate agar residues, and transferred to pots containing a sterile substrate mixture of 2/3 peat and 1/3 perlite. The containers were covered with transparent plastic domes to maintain high humidity for approximately two weeks. After gradual removal of the covers, plantlets were grown under ambient conditions until full acclimatization.

2. Molecular analyses

2.1. Genomic DNA extraction

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Genomic DNA was extracted from leaf tissues collected from ten regenerated plantlets. Approximately 50 mg of fresh tissue were processed using the Maxwell™ 16 Instrument (Promega), following the manufacturer's protocol. The automated system allows simultaneous extraction of up to 16 samples, generating genomic DNA of high purity (~99%), suitable for molecular analyses.

2.2. PCR amplification using SCoT markers

The PCR amplification was performed using the GoTaq® Green Master Mix 2× (Promega), containing GoTaq® DNA Polymerase, dNTPs, $MgCl_2$, and reaction buffer at optimized concentrations (table 2).

Table 2. Composition of the PCR reaction mixture (25 µl total volume):

Component	Volume (1×)
Green Master Mix	12.5 µl
Sterile distilled water	9 µl
Primer (10 pmol/µl)	1.5 µl
Genomic DNA (50–100 ng)	2 µl

Three SCoT primers (T4, T5, T7) targeting the start codon (ATG) region were used in this experiment.

2.3. PCR reaction conditions

Amplifications were carried out in a Corbett thermocycler using the following program:

- Initial denaturation: 94°C for 5 min
- 35 cycles of:
  - Denaturation: 94°C for 1 min
  - Annealing: 46–51°C for 1 min (depending on primer)
  - Extension: 72°C for 3 min
- Final extension: 72°C for 7 min
- Final hold: 4°C

PCR products were stored at 4°C until electrophoresis.

2.4. Analysis of PCR products by agarose gel electrophoresis

Amplified fragments were separated by electrophoresis on 2% agarose gels prepared in 1× TAE buffer and stained with ethidium bromide. Gels were run at 100 V for 60–90 minutes and visualized under UV light using a gel documentation system. Band size was estimated based on a molecular weight marker, following the classical principles of DNA migration in agarose matrices [16].

Results and Discussion

1. Experimental results regarding *in vitro* culture

1.1. In Vitro Regeneration

In our study, the appearance of in vitro shoots 20 days after culture initiation was a significant moment in the development of cell cultures (figure 1). This phenomenon indicates that, during this period, the explant cells began to develop and differentiate into primary plant structures, demonstrating the totipotency of plant cells in an appropriate culture medium.

Figure 1. Plantlets obtained through organogenesis in Parma violet.

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1.2. In vitro multiplication through direct organogenesis

At eight weeks after the initiation of the culture, we performed shoot counts and measurements of shoot length (figure 2). These observations and measurements can be used to monitor and select plants with higher multiplication rates and more vigorous growth. Thus, this information is valuable for optimizing and improving the in vitro culture protocol, contributing to the development of a uniform and healthy plant population. The results of these measurements provide insight into the growth and developmental rate of the in vitro plants, serving as an important indicator of the efficiency of the cell cultures. The number and length of shoots reflect the plant response to in vitro culture conditions, enabling the evaluation of the success of the regeneration and development process. Table 1 presents the experimental data regarding the number of shoots and their height, obtained after testing three different hormonal balances. The results indicate a significant variation in the efficiency of plant regeneration depending on the combination of growth hormones used.

Figure 2. Plantlets after 8 weeks

Table 1. Experimental results

No.	Experimental Variant	Number of Explants / Hormonal Variant	Shoot height (cm)	Number of regenerants / Explant
1	V1	20	1.10 ± 1.1	8.1 ± 0.6
2	V2	20	2.15 ± 0.9	13.5 ± 0.7
3	V3	20	3.25 ± 1.2	21.4 ± 1.7

Note: The results are expressed as mean ± standard deviation

The table shows a clear and consistent increase in both shoot height and number of regenerants per explant across the experimental variants (V1–V3). Variant V1 exhibits the lowest values, indicating a weak regenerative response under its hormonal conditions. In V2, both parameters improve noticeably, suggesting a more effective hormonal combination. Variant V3 provides the best outcomes, with shoot height tripling compared to V1 and more than 21 regenerants per explant, demonstrating that this variant represents the optimal experimental formulation for promoting regeneration and vegetative growth under the tested conditions.

The results obtained highlight the importance of optimizing the culture medium to maximize the efficiency of plantlet regeneration. The experimental variant V3, with an optimized hormonal composition, demonstrates that precise adjustment of culture conditions can lead to significant improvements in micropropagation processes.

According to the data, the highest number of shoots per explant was achieved when explants were cultivated on MS medium supplemented with 2 mg/L BA (benzyladenine) and 1 mg/L NAA (naphthaleneacetic acid). This suggests that, under the given experimental conditions, the combination of BA and NAA is the most effective in supporting direct organogenesis-based regeneration. Figure 3 provide a visual representation of these results, clearly illustrating the differences in the number of shoots obtained per explant depending on the hormonal combination used.

The synergistic effect between the auxin NAA and the cytokinin BA appears to have a strong stimulatory impact on regeneration through direct organogenesis. Auxins and cytokinins play complementary roles in cell division and differentiation, and combining them in optimal proportions can accelerate and enhance plant regeneration.

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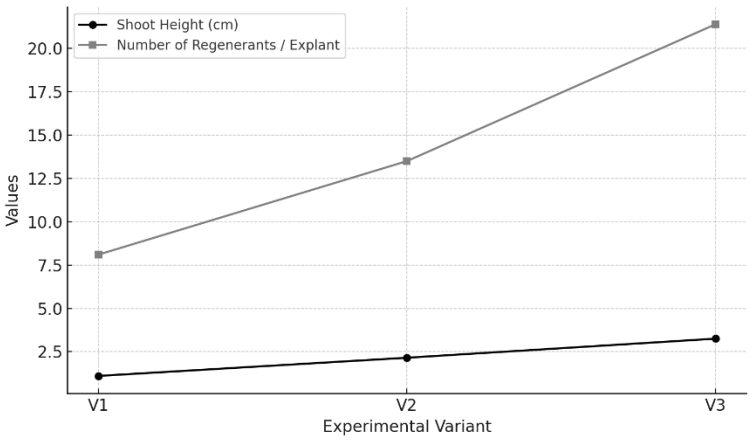

Figure 3. Regenerants and shoot length

1.3. Experimental results on plantlet rooting

According to the results obtained in this experiment, shoot rooting occurred within a relatively short period, between 2 and 3 weeks. Most of the shoots inoculated on the culture medium developed roots, indicating that root formation was an easy and rapid stage within the in vitro regeneration protocol used in this study. The shoots that did not produce roots during this stage were successfully rooted during acclimatization.

1.4. Experimental results on plantlet acclimatization

Following the in vitro regeneration process, approximately 50 plants were successfully acclimatized. Acclimatization is an essential step in plant micropropagation, representing the transition of regenerated plants from the controlled conditions of tissue culture to the external environment. In this experiment, acclimatization was carried out by transferring the plants into soil-filled containers, thereby providing a suitable transitional environment (figure 4).

Figure 4. Acclimatized plants

2. Experimental results on the molecular evaluation of somaclonal variation using SCoT markers

The results obtained in this study highlighted the presence of significant genetic stability in 10 violet regenerants using three different SCoT primers (T4, T5, T7). The similar amplification profiles obtained among the regenerants indicate a high degree of genetic homogeneity, suggesting that somaclonal variation was minimal in this case. This type of genetic stability is particularly important in breeding and conservation research, as it ensures the preservation of specific genetic characteristics and prevents the occurrence of undesirable variations.

The results obtained with primer T4 showed that the generated amplicons were similar in all 10 regenerants, with no differences observed between them and the donor plant. This indicates that all regenerants inherited the same gene sequence amplified by primer T4, reflecting a high level of genetic uniformity within the regenerated material. Figure 5 illustrates these findings, confirming the genetic stability of the regenerants compared to the donor plant.

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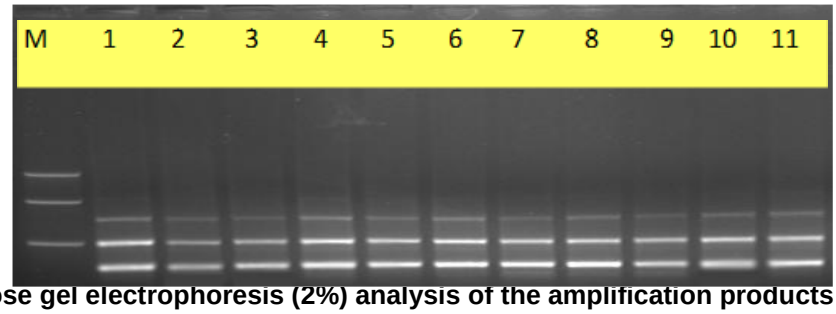

Fig. 5. Agarose gel electrophoresis (2%) analysis of the amplification products obtained with primer T4 Legend: M – molecular marker; 1–10 – regenerants; 11 – explant donor plant.

The absence of genetic differences between the regenerants and the donor plant suggests that the in vitro regeneration process was highly genetically stable. This is essential when the preservation of specific genetic traits of the donor plant is desired. The regeneration method used demonstrated remarkable efficiency in maintaining genetic integrity, which is crucial for the reproduction and conservation of genetic material. The other two primers included in the study also confirmed genetic stability.

These findings have important implications for the reproduction and conservation of plant genetic material. The genetic stability maintained during the in vitro regeneration process is essential to ensure that the desired characteristics of the donor plant are preserved in the regenerants.

Conclusions

The in vitro regeneration protocol developed in this study proved to be highly effective, enabling the consistent production of shoots and viable plantlets across all tested hormonal variants.

The combination of 2 mg/L BA and 1 mg/L NAA (Variant V3) produced the best results, yielding the greatest number of regenerants and the highest shoot elongation, demonstrating a strong synergistic effect between cytokinin and auxin in promoting direct organogenesis.

Rooting occurred rapidly on hormone-free MS medium, with the majority of shoots developing well-formed root systems within 2–3 weeks, confirming the good physiological condition of the regenerated material.

The acclimatization procedure was successful, with approximately 50 plantlets surviving and adapting to ex vitro conditions, highlighting the practical applicability of the protocol for large-scale multiplication.

Molecular analysis using SCoT markers (T4, T5, T7) revealed no detectable genetic polymorphisms among the regenerants, indicating high genetic stability and minimal somaclonal variation during the regeneration process.

The demonstrated genetic fidelity confirms that direct organogenesis is a reliable method for clonal propagation of *Saintpaulia ionantha*, ensuring the preservation of desirable traits from the donor plant.

Overall, the integrated approach combining morphogenic evaluation and molecular analysis validates this micropropagation system as efficient, reproducible, and suitable for breeding programs, commercial propagation, and germplasm conservation.

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