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SOMATIC EMBRYOGENESIS AND BIOREACTOR SCALE-UP OF BANANA HYBRID FHIA-18 (AAAB)

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ABSTRACT

In order to regenerate plants and conduct somatic embryogenesis in FHIA-18 (AAAB) bananas, the researchers in this work used embryogenic cell suspensions made from male flower tissues. The packed cell volume (PCV) technique was used to evaluate cell growth while cell suspensions were grown in a liquid medium based on MS. Plant growth regulators and carbohydrates in SH medium induced somatic embryos, whereas 6-BA and IAA in MS medium optimised multiplication. Analyses conducted in bioreactors involving dissolved oxygen (DO_2) and pH control showed that the former synchronised maturation and the latter increased somatic embryo proliferation. The germination process was carried out in RITA temporary immersion systems, and at the ideal inoculation density, the germination rate reached 89.3 percent. Regeneration was boosted using a scaled-up 10-liter RITA system, which allowed each bottle to produce up to 1200 plants. After being acclimated, the regrown plants were then sent to field settings to be tested further. This study provides a scalable technique for banana propagation by demonstrating the efficacy of short-term immersion systems in enhancing somatic embryogenesis and plant regeneration.

Keywords: *Bioreactor, Somatic Embryogenesis etc.*

INTRODUCTION

For economies and food supplies worldwide, but especially in the tropics and subtropics, the banana (Musa spp.) is an indispensable fruit crop. Its economic importance, adaptability, and nutritional value make it an ideal crop for widespread cultivation. Because of their increased tolerance to pests, diseases, and environmental stress, hybrid banana cultivars like FHIA-18 (AAAB) have become more popular. A major step forward in banana breeding has been achieved with the creation of this hybrid by the Fundación Hondureña de Investigación Agrícola (FHIA). It combines favourable agronomic qualities with improved adaptation to a wide range of growing environments. However, there are a number of problems with traditional techniques of banana hybrid propagation, such as suckering and tissue culture, such as poor multiplication rates, somaclonal variances, and expensive production costs. Somatic embryogenesis (SE) and bioreactor scale-up provide a potential solution to these restrictions, allowing for the efficient and large-scale production of high-quality banana plants.

Somatic Embryogenesis in Bananas

Producing genetically homogeneous and disease-free plantlets from somatic cells is made possible using the sophisticated in vitro propagation method known as somatic embryogenesis (SE). During SE, bipolar

formations that resemble zygotic embryos can grow into fully formed plants, in contrast to organogenesis that results in the immediate creation of shoots. Embryo maturation, embryogenic callus development, callus induction, germination, and plantlet conversion are the several crucial steps involved in this process. Applying SE to several Musa species has been a success, showing that it might be useful for commercial micropropagation and improving genetics.

Explants of immature zygotic embryos, highly proliferative meristematic tissues, or immature male flowers are usually needed for the production of somatic embryos in bananas. To initiate embryogenic callus development, growth regulators including 2,4-Dichlorophenoxyacetic acid (2,4-D), Thidiazuron (TDZ), and Naphthaleneacetic Acid (NAA) are essential. Improving the composition of culture media—including the ratio of macro- and micronutrients, vitamins, and organic additives—is crucial for achieving the best possible embryogenic potential. Somatic embryogenesis can be made more efficient by exposing the embryos to certain elicitors, osmotic stress, or desiccation therapies.

Despite the benefits, SE in bananas is frequently accompanied by difficulties such lengthy induction durations, poor embryogenic competence, and resistance in certain genotypes. So, to make SE-based propagation more efficient and scalable, we need to standardise methods and incorporate automated technologies like bioreactor systems.

Enhancing Bioreactor Performance for Large-Scale Propagation

Solid or semi-solid culture medium are the backbone of traditional micropropagation methods, however they are labour-intensive and can't handle plant production on a big scale. By automating culture conditions, lowering contamination concerns, and increasing cost-effectiveness, bioreactor systems provide a more efficient option. For banana micropropagation, the use of CIBs and TIBs has been widespread because they greatly improve plantlet quality and multiplication rates.

To keep explants from becoming hyperhydrated, TIB systems submerge them in liquid culture fluid at regular intervals, which improves nutrient absorption. When compared to more traditional techniques of plant culture, the RITA® and SETIS® bioreactors provide far better results for shoot growth and somatic embryo conversion in Musa spp. Conversely, CIB systems, such stirred-tank and air-lift bioreactors, provide for constant aeration and nutrient exchange, enabling high-density growth conditions that are well-suited for large-scale uses.

Precise optimisation of aeration rate, agitation speed, pH regulation, dissolved oxygen levels, and nutrient replenishment is necessary for the bioreactor-based scale-up of banana hybrid FHIA-18. Another way to improve reproducibility and productivity is to automate culture monitoring with sensors and real-time analytics. Commercial banana propagation that makes use of bioreactor technology has several advantages, such as faster plantlet acclimatisation rates, more efficient multiplication, and lower production costs.

REVIEW OF LITERATURE

Natarajan Nandhakumar et.al. (2018) This study aimed to increase banana variety production through somatic embryogenesis, focusing on Grand Naine (Musa AAA, Cavendish) and Rasthali (Musa AAB, Silk). The impact of male flower bud position, amino acid supplements, and somatic embryogenesis induction on field performance was investigated. Juvenile male flower buds (6th–8th bract whorl) showed superior callus

induction, while glutamine (400 mg/L) with sucrose-maltose (20:20 g/L) increased somatic embryo production tenfold. Dehydrating cotyledonary-stage embryos improved germination rates, and ISSR markers confirmed genetic fidelity. ECS-derived plantlets exhibited morphological traits similar to control plants in field trials, demonstrating the method's potential for large-scale micropropagation and genetic modification of economically important banana cultivars.

Lekshmi, R.S. et. al. (2017) A rapid and simple method was developed for somatic embryogenesis in banana cv. Nendran (AAB) using immature male flowers (IMF). Embryogenic callus (30% response) formed on MS medium with BA (0.05–0.50 mg/L) and picloram (0.50–2.00 mg/L). Ascorbic acid (20 mg/L) and Gelrite (0.45%) reduced phenolic interference. Embryogenesis (33.3%–60%) was induced on MS medium with BA (2 mg/L) and IAA (0.5 mg/L), with germination rates of 60%–80% in 72 hours. Under 14h light/8h dark, embryos transferred to MS medium with BA (2 mg/L) and NAA (1 mg/L) showed 100% conversion to plantlets. This efficient six-month process eliminates the need for intermediate liquid cultures.

Rajvir Singh et.al. (2015) An efficient in vitro propagation method for William Hybrid banana using suckers was developed. BAP (5 mg/L) induced the highest shoot bud proliferation, yielding 5–10 shoots per explant. Repeated subculturing enhanced multiplication, while rooting was achieved on ½ strength MS with 1 mg/L IBA. Hardened plantlets successfully established in soil, demonstrating the method's effectiveness for large-scale propagation.

Lalremsiami Hrahsel and Robert Thangjam (2013) Plant tissue culture is a widely accepted method for mass propagation of various plant species, including bananas. In India, micropropagation units produce millions of disease-free, genetically uniform plantlets to meet demand. Since conventional propagation is inefficient for large-scale banana production, tissue culture remains the only viable method. This study explores tissue culture techniques for commercial banana production in Mizoram.

Smitha Prasanna Divakaran and Ashalatha Sankarankutty Nair (2011) Shortened Version: Embryogenic callus regeneration was studied in four diploid banana cultivars (Matti, Sannachenkadali, Chingan, Njalipoovan) using bract explants on MS medium with TDZ (0.045–9.00 µM). Somatic embryo formation increased in Matti, Chingan, and Njalipoovan with biotin (8.18–16.37 µM), while Sannachenkadali showed maximum embryos with glutamine (6.84 µM). Regenerated plants exhibited minimal somaclonal variation and achieved 90% survival in soil.

OBJECTIVES OF THE STUDY

Following are the main Objective of this study: -

1. To optimize bioreactor-based somatic embryogenesis for large-scale banana propagation.
2. To develop an efficient bioreactor system for large-scale plant propagation.

HYPOTHESIS

Following are the main hypothesis of this study: -

H_1: There is a significant enhancement in banana propagation through bioreactor-based somatic embryogenesis.

H_2: There is a significant impact of an optimized bioreactor on plantlet growth and uniformity.

MATERIALS AND METHODS

This study established FHIA-18 (AAAB) cell suspensions from six-month-old embryogenic tissue. Suspensions were subcultured in MS liquid medium and filtered every 15 days. Somatic embryos were induced in SH medium with PGRs and sugars, assessing inoculation densities. Multiplication of globular embryos used MS medium with 6-BA and IAA under different densities. Cultures were maintained at 27°C in the dark with orbital shaking. Bioreactors analyzed DO_2 and pH effects, while germination was tested in RITA systems with MS medium and varying inoculation densities, scaling up to 10-L bottles with three daily immersions.

RESULTS

Embryogenic cell suspensions of the hybrid cultivar FHIA-18 (AAAB) were established from male flower-derived tissue cultured for six months. These suspensions were subcultured in liquid Murashige and Skoog (MS) medium with supplements, filtered every 15 days, and maintained at 27°C in the dark under orbital shaking. Growth was evaluated using the packed cell volume (PCV) method.

Somatic embryogenesis was induced in Schenk and Hildebrandt (SH) medium supplemented with plant growth regulators (PGRs) and sugars, using varying inoculation densities. Multiplication of globular embryos was carried out in MS medium with 6-BA and IAA at different densities. The effects of dissolved oxygen (DO_2) and pH control were analyzed in bioreactors under different DO_2 levels.

For germination, somatic embryos were cultured in RITA temporary immersion systems with MS medium under controlled light and temperature conditions, using different inoculation densities. The RITA system was successfully scaled up using 10-L autoclavable bottles. The study confirmed the successful establishment of embryogenic cell suspensions, characterized by dense cytoplasmic content, high cell division, and optimal growth and multiplication conditions.

Table: 1 Impact of inoculation density on somatic embryo formation and total weight in FHIA-18 (AAAB) banana

Initial inoculum (mg)a	Average number of globular stage somatic embryos per ml of cell suspension		Total weight (mg) of somatic embryos
of cell aggregates	15 days	30 days	
50	248±22	448±20	127.5±3.1
100	780±25	1883±23	255.0±2
250	345±24	648±24	157.5±3

500	52±24	186±24	48.0±2

Values are presented as mean ± standard error. Measurements were conducted in 25 mL of modified SH medium using four 250-mL Erlenmeyer flasks.

Table: 2 Inoculation density effect on FHIA-18 somatic embryo multiplication in liquid medium

Initial density of inoculum SE per 25 ml	Total fresh weight of somatic embryos at 30 days	Total number of somatic embryos at 60 days
(g)	(g)	
0.2	18.40±0.25	500±49
0.4	16.22±0.18	4 200±34
0.6	25.20±0.23	16 700±52

Values are presented as mean ± Standard Error (n=5).

A: MS medium supplemented with 0.3 mg/L 6-BA, 2.0 mg/L IAA, and 30 g/L sucrose in a 25 mL volume within a 250 mL Erlenmeyer flask.

Table: 3 Effect of DO_2 on somatic embryo growth in FHIA-18 bioreactors

Stages of somatic	Treatments		General average±
embryogenesis	80.0%	80.0% first 7 days	Standard error
	(throughout)	then 40.0% until the	
		end	
Globular embryos per litre	48767 a	31333 b	40050.0±245.4
Scutellar and coleoptilar	10253 b	14302 a	12277.5±79.5
embryos per litre			
Mature embryos per litre	9061 b	20888 a	14974.5±123.4
Total embryos per litre	68081 a	66523 a	67302±445.9

Different letters in a row indicate significant differences based on Dunnett's C-test ($p \leq 0.05$). Globular somatic embryos accumulated at the base of the Erlenmeyer flask.

Among the four initial cell densities studied for somatic embryo formation in liquid media, the best results were obtained with an initial inoculum of 100 mg (Table 1), yielding 1,883–1,906 globular embryos per mL

of suspension after 30 days of culture. Similar findings have been reported in other cultivars using semi-solid medium (Bieberach, 1995; Cote et al., 1996; Grapin et al., 1998).

Somatic embryo diameters in the cell suspensions ranged from 0.5 to 1.2 mm, with an average size of 0.86 ± 0.25 mm. Their weight varied between 0.65 and 0.90 mg, depending on the developmental stage, with an estimated average weight of 0.76 ± 0.16 mg. The best results for embryo multiplication in modified MS culture medium (repetitive or secondary embryogenesis) were achieved under optimized conditions.

Table: 4 Effect of pH control on FHIA-18 somatic embryo growth in the first 7 days

Stages of somatic	Treatments	General average±
embryogenesis	80.0% of DO2 controlled 80.0% of DO2 and and pH pH free	Standard error
Globular embryos per litre	123 270 a 45 073 b	84 171.5±515.7
Scutellar and coleoptilar	15 864 a 7 780 b	11 822±74.3
embryos per litre		
Mature embryos per litre	2216 b 6512 a	4364±27.4
Total embryos per litre	141 350 a 59 365 a	100 357.5±614.8

Different letters in one row represent significant differences by the Dunnett's C-test, $p \leq 0.05$.

Table 5: Somatic embryo germination (%) under different conditions at 40 days

System type	Initial quantity of somatic embryos (g)	Number of plants formed	Germination (%)
RITA	1.0	184	45.5
RITA	1.5	379	58.0
RITA	2.0	748	89.3
RITA	3.0	525	77.0
Culture flask	20 SE per flask	7	26.6

A RITA system with a 500 mL capacity containing 200 mL of culture medium was used. The culture medium consisted of MS medium with vitamins according to Morel and Wetmore (1951), supplemented with 0.5 mg/L 6-BA, 2.0 mg/L IAA, and 30.0 g/L sucrose. Results showed that an initial density of 0.6 g in 25 mL of liquid medium led to a 42-fold increase in somatic embryos after 30 days (Table 2). Notably, repetitive embryogenesis occurred even without an exogenous auxin, a process known as autoembryogenesis, sometimes referred to as proliferation or mass propagation (Merkle et al., 1995).

In bioreactor cultures, maintaining a high and constant dissolved oxygen (DO_2) concentration of 80.0% resulted in a greater proportion of globular somatic embryos at the end of the culture period (48,767 SE/L), representing 71.63% of the total embryos, while 13.30% matured (Table 3). However, reducing the DO_2 concentration after 7 days led to an increase in mature somatic embryos (31.39%) and a decrease in globular embryos (47.10%). Similar findings were reported by Mavituna and Buyukalaca (1996) in Capsicum annuum L. cv. Ace, where oxygen uptake rates varied depending on the stage of embryogenesis, with the highest rate in embryogenic suspension cultures and the lowest during embryo maturation.

The effect of pH control on embryogenesis was evaluated at 80.0% DO_2 during the first 7 days under two conditions: one with free pH and another with pH fixed at 3.80. The pH-controlled treatment resulted in greater uniformity in embryo size and development (Figure 1A), yielding a higher number of somatic embryos per liter of culture medium (141,350 vs. 59,365, Table 4). In this treatment, 87.2% of embryos remained at the globular stage, confirming the beneficial effect of high DO_2 concentrations on somatic embryo multiplication and pH control on synchronizing maturation. Embryo size varied between treatments, with large embryos measuring 1.53 mm (pH-controlled) vs. 2.45 mm (free pH) and small embryos measuring 0.39 mm (pH-controlled) vs. 0.43 mm (free pH). Only 1.5% of embryos matured in the pH-controlled bioreactor, compared to 10.9% in the free pH condition, likely due to the higher density of globular embryos, which may have hindered further development. Similar observations were made by Osuga et al. (1993) and Shigeta et al. (1996).

A portion of somatic embryos was transferred to temporary immersion systems (RITA). After 30 days, germination reached 89.3% using 2.0 g of somatic embryos per 200 mL of culture medium, outperforming other treatments and controls on semi-solid medium (Table 5). Notably, temporary immersion systems reduced embryo oxidation, supporting findings by Bieberach (1995). After just 7 days, a significant proportion of embryos had already initiated germination, similar to results observed in other temporary immersion systems.

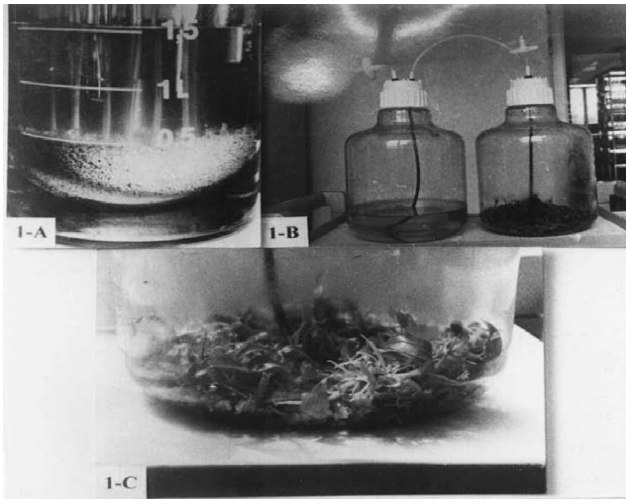

Figure: 1 (A) Somatic embryos (80% DO2, 25 days). (B) Temporary immersion (10-L bottle). (C) Regenerated plants.

A 10-L immersion system (Nalgene Co.) achieved germination rates of 65.5–73.0%, producing 800–1200 plants per bottle (Figure 1B). Temporary immersion ensured more consistent and synchronized somatic

embryo development, aligning with findings in Hevea brasiliensis (Etienne et al., 1997) and Citrus deliciosa (Cabasson et al., 1997).

Regenerated plants from temporary immersion and solid media were regularly transferred to MS medium without growth regulators, completing their development within two months. Subsequently, 1000 plants were transferred to polyurethane boxes alongside an equal number of conventionally micropropagated plants for field study. Given the challenges of somaclonal variation in micropropagation and somatic embryogenesis, this new propagation protocol must be compared with standard banana micropropagation techniques to assess genetic stability.

DISCUSSION

The study successfully established and optimized embryogenic cell suspensions of the FHIA-18 (AAAB) banana using male flower-derived tissue, demonstrating the viability of liquid culture systems for large-scale propagation. The research highlighted the significance of Murashige and Skoog (MS) and Schenk and Hildebrandt (SH) media, supplemented with appropriate plant growth regulators, in facilitating somatic embryo induction. The study further examined the role of inoculation density in embryo formation and multiplication, identifying an initial 100 mg inoculum as the most effective, yielding the highest number of globular embryos. Optimized culture conditions, particularly the regulation of dissolved oxygen (DO_2) and pH, significantly influenced embryo growth and maturation. Higher DO_2 concentrations (80%) favored the formation of globular embryos, whereas reducing DO_2 levels after seven days promoted embryo maturation. Furthermore, pH control at 3.80 resulted in improved embryo uniformity and multiplication, emphasizing the importance of synchronized environmental parameters in somatic embryogenesis. The study also explored the role of temporary immersion systems (TIS), particularly RITA, in enhancing somatic embryo germination. The most effective germination rates (89.3%) were achieved when 2.0 g of somatic embryos were cultured in 200 ml of MS medium. Scaling up the RITA system to 10-L autoclavable bottles demonstrated its potential for mass propagation, successfully producing up to 1200 plants per container. The advantages of temporary immersion systems over conventional culture methods were evident, as they significantly reduced embryo oxidation and improved synchronization, resulting in higher-quality plantlets. Following germination, regenerated plants were transferred to hormone-free MS medium, where they exhibited robust development before being acclimatized in field conditions. The study's findings suggest that the developed somatic embryogenesis system offers a scalable, efficient, and high-yield propagation method for bananas, presenting an alternative to conventional micropropagation techniques. However, ensuring genetic stability remains a critical factor in commercial application. The potential for somaclonal variation necessitates comparative genetic assessments between plants produced through this system and those propagated using traditional micropropagation methods. Further research focusing on molecular and cytogenetic analyses will be essential to confirm the clonal fidelity of plants generated via this method, ensuring its long-term viability for banana breeding and large-scale agricultural production.

CONCLUSION

The study will establish an optimized protocol for the large-scale propagation of FHIA-18 (AAAB) bananas through somatic embryogenesis, demonstrating the potential for efficient plant multiplication. Future

research will focus on refining bioreactor conditions, particularly dissolved oxygen (DO_2) levels and pH control, to enhance embryo synchronization and development. The scalability of the RITA immersion system will enable higher germination rates, ensuring consistent and uniform plant regeneration. Long-term studies will assess the genetic stability of regenerated plants compared to conventional micropropagation techniques. These advancements will contribute to the development of improved propagation strategies for banana cultivation, supporting sustainable agricultural practices.

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