La granja:
Revista de
Ciencias de la Vida

pISSN:1390-3799; eISSN:1390-8596

https://doi.org/10.17163/lgr.n43.2026.07

Review / Reseña bibliográfica

Agricultural Sciences

In vitro propagation of banana (*Musa* spp.) by somatic embryogenesis

Propagación *in vitro* de banano (*Musa* spp.) por embriogénesis somática

Jessenia Lucero-Murillo *, Jorge Manzano-Torres, Iliana Loaiza-Maldonado and Yamile Orellana-García

Instituto Superior Tecnológico Ismael Pérez Pazmiño, Machala, Ecuador.

*Corresponding author: jesylu_@hotmail.es

Article received on May 5th, 2022. Accepted, after review, on November 23th, 2022. Published on March 1st, 2026.

Abstract

The banana is a fruit of great consumption worldwide due to its high nutritional value and is a source of economic income for producing countries. However, the susceptibility of cultivars to pests and diseases, and the complexity of plant regeneration due to high levels of ploidy and sterility, hinder the process of plant propagation. Somatic embryogenesis (SE), through embryogenic cells, constitutes an effective tool for the mass propagation of banana plants due to high multiplication coefficients and low production costs. The purpose of this article is to describe the processes of *in vitro* propagation of banana plants (*Musa* spp.) through a bibliographic review of the applications of somatic embryogenesis in micropropagation and genetic improvement. A systematic review was conducted in specialized search engines and databases through three stages: planning, execution, and summary.The most relevant results indicate that SE represents an alternative for plant multiplication due to its enormous potential for tissue regeneration in a short period. However, the risk of somaclonal variation has limited its expansion on a commercial scale. On the other hand, SE is being used in studies related to the genetic transformation of plants. The susceptibility of the crop to diseases such as *M. fijiensis*, *F. oxysporum* f. sp. *cubense* (Foc R4T), and banana streak virus (BSV) has encouraged the development of resistant varieties through embryogenic cell culture and the use of protoplasts. Similarly, the application of gene transfer or gene editing techniques has made it possible to obtain new varieties with resistance or tolerance to the main crop diseases.

Keywords: *Callus*, Genetic variability, *In vitro*, *Musa*, Somatic embryogenesis.

Resumen

El banano es una fruta de gran consumo a nivel mundial por su alto valor nutritivo y es fuente de ingresos económicos para los países que se dedican a la producción de la fruta. Sin embargo, la susceptibilidad de los cultivares a plagas y enfermedades evidencia la baja producción en el cultivo. Asimismo, los altos niveles de ploidía y esterilidad dificultan el proceso de propagación de plantas. La embriogénesis somática (ES), a través de células embriogénicas, constituye una herramienta eficaz para la propagación masiva de plantas de banano por los altos coeficientes de multiplicación y bajos costos de producción. El presente artículo tiene como finalidad describir los procesos de propagación *in vitro* de plantas de banano (*Musa* spp.), mediante una revisión bibliográfica de las aplicaciones de embriogénesis somática en la micropropagación y mejoramiento genético. Para lo cual se realizó una revisión sistemática en buscadores especializados y bases de datos, a través de tres etapas: planificación, ejecución y resumen. Los resultados más relevantes indican que la ES representa una alternativa para la multiplicación de plantas, por el gran potencial para la regeneración de tejidos en un corto periodo de tiempo. Sin embargo, el riesgo de variación somaclonal ha limitado su expansión a escala comercial. Por otro lado, la ES está siendo empleada en estudios relacionados con la transformación genética de plantas. La susceptibilidad del cultivo a enfermedades como *M. fijiensis*, *F. oxysporum* f. sp. *cubense* (Foc R4T) y virus rayado del banano (BSV), ha incentivado el desarrollo de especies resistentes a través del cultivo de células embriogénicas y uso de protoplastos. De igual forma, la aplicación de técnicas de transferencia de genes o edición genética ha permitido obtener nuevas especies con resistencia o tolerancia a las principales enfermedades del cultivo.

Palabras clave: *Callos*, Embriogénesis somática, *In vitro*, *Musa*, Variabilidad genética.

Suggested citation: Lucero-Murillo, J., Manzano-Torres, J., Loaiza-Maldonado, I. and Orellana-García, Y. (2026). *In vitro* propagation of banana (*Musa* spp.) by somatic embryogenesis. La Granja: Revista de Ciencias de la Vida. Vol. 43(1):128-144. https://doi.org/10.17163/lgr.n43.2026.07.

Orcid IDs:

Jessenia Lucero-Murillo: https://orcid.org/0000-0001-6723-8249
Jorge Manzano-Torres : https://orcid.org/0000-0002-4652-8877
Iliana Loaiza-Maldonado: https://orcid.org/0000-0003-2703-4887
Yamile Orellana-García: https://orcid.org/0000-0001-6956-8276

1 Introduction

Banana plants produce fruits that are highly valued in tropical and subtropical regions worldwide (Tran et al., 2016). Bananas rank second among fruit crops, with a production of 115.7 million tons, and constitute one of the crops with the greatest impact on the economies of exporting countries (FAO, 2020). However, classical propagation methods of edible *Musa* spp. are complex due to levels of ploidy and sterility (Simoníková et al., 2022). Somatic embryogenesis (SE) is a tool for the mass propagation of high-yielding genotypes (Tran et al., 2016). This technique consists of selecting zygotic embryos, proliferating meristems or scalps, as well as male and female flowers (Ahmed et al., 2014). The selected material initiates a process of embryogenic callus induction, followed by the establishment of embryogenic cell suspensions, the formation of somatic embryos, maturation, and germination; subsequently, plants enter the acclimatization process before being transferred to open field conditions (Escobedo-Gracia et al., 2016; Taco et al., 2026).

Despite the high plant regeneration capacity, this specialized technique is mainly directed toward genetic improvement rather than commercial propagation, due to the risk of somaclonal variation, in comparison with plants obtained through organogenesis (Galán et al., 2018). Somatic cell suspension cultures constitute an alternative for banana plant improvement (Liu et al., 2017). However, the use of protoplasts obtained through embryogenesis from cell suspension cultures (ECS) has been frequently employed because of their high yield, high activity, ease of operation, and wide adaptability (Wu et al., 2020). Current genetic transformation methods include *Agrobacterium*-mediated transformation (Kovács et al., 2013), particle bombardment (Vishnevetsky et al., 2011), and CRISPR/Cas9 (Wu et al., 2020).

These approaches have enabled genetic improvements in cultivars susceptible to diseases (Shivani et al., 2017), such as the development of plants resistant to banana streak virus (BSV) and *Mycosphaerella fijiensis* (Tripathi et al., 2019; Kovács et al., 2013). Despite the development of improved banana plants and the achievement of ideal plant architecture, sustained immune responses are consistently accompanied by yield reductions (Wang et al., 2021). Therefore, it is necessary to conduct studies on the *Musa* spp. genome, perform gene-editing experiments, and establish *in vitro* culture protocols that allow optimization of banana plant transformation processes. Consequently, this review article aims to describe the *in vitro* propagation processes of banana plants (*Musa* spp.) through a bibliographic review of the applications of somatic embryogenesis in micropropagation and genetic improvement.

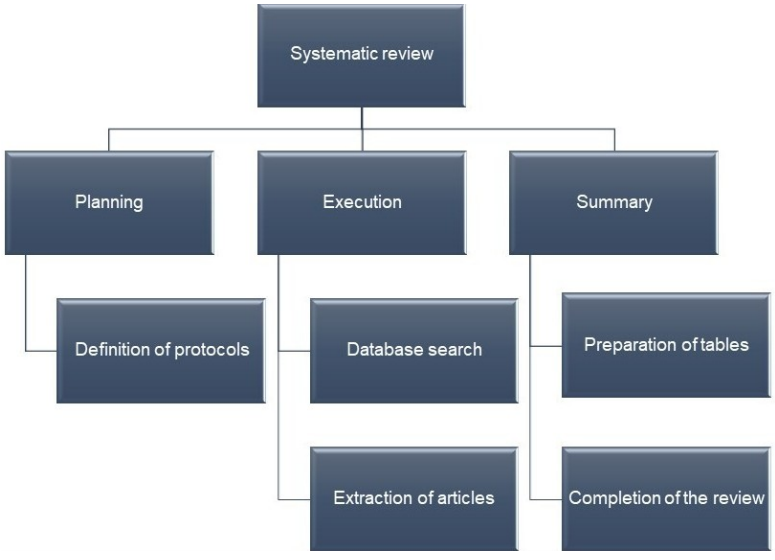

Figure 1. Methodological process of critical screening of scientific articles.

2 Methodology

The systematic review was conducted through three stages (Moreno et al., 2018): planning, execution, and synthesis (Figure 1). The selected information corresponds to articles published over the last 10 years, with some exceptions included due to their relevance to the literature review.

During the planning stage, a protocol was developed to be followed throughout the entire review process. This protocol included information such as article title, authors, objective, keywords, research sources, inclusion and exclusion criteria, and type of study. In the execution stage, bibliographic information was obtained from specialized databases such as ResearchGate, Semantic Scholar, Google Scholar, Springer, SciELO, ScienceDirect, Frontiers, and PubMed. Automated searches were conducted based on titles, keywords, and abstracts (Figure 2). The synthesis stage comprised the preparation of figures and tables, as well as the writing of the manuscript.

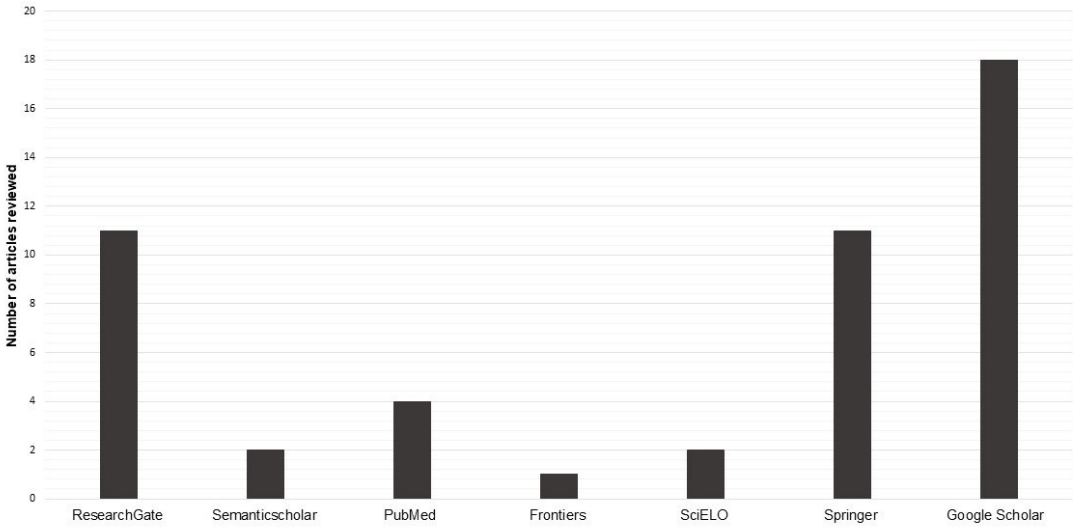

Figure 2. Systematic review in specialized search engines.

3 *In vitro* culture in banana

Banana is a monocotyledon belonging to the genus *Musa* spp. and is among the most important and widely distributed crops in humid tropical and subtropical regions worldwide (Escobedo-Gracia et al., 2016). In Ecuador, banana cultivation represents the most economically relevant agricultural activity (Capa Benítez et al., 2016). In addition, the country is the world's leading exporter, supplying markets such as the European Union, the United States, Asia, among others (MAGAP, 2016). Currently, diploid and triploid hybrids originating from natural intraspecific crosses (2n = 2x = 22 chromosomes) are cultivated, derived from *Musa acuminata* Colla (genome A) and *Musa balbisiana* Colla (genome B) (Martin et al., 2020). However, one of the main limitations of banana cultivation is polyploidy and vegetative parthenocarpy (Escobedo-Gracia et al., 2016). Vegetative propagation through natural shoots known as suckers or buds from the rhizome fails to meet the demand for elite genotypes required for crop establishment (Lohidas and Sujin, 2015).

In vitro propagation involves excising a small fragment of the plant, known as an explant, and culturing it under aseptic conditions with macronutrients, micronutrients, carbohydrates, vitamins, growth regulators, and occasionally amino acids, all developed under controlled environments (Ahmed et al., 2014). This technique comprises four stages:

establishment, multiplication, rooting, acclimatization, and hardening (Rodríguez, 2013).

In banana, *in vitro* propagation is carried out using apical meristems extracted from the rhizome, as these exhibit longitudinal growth as a result of cellular totipotency (Ahmed et al., 2014). To ensure normal development, meristems must be established in an optimal culture medium composed of inorganic salts, organic compounds, complex natural preparations, and inert support materials (Anbazhagan et al., 2014). The most widely used culture medium for *in vitro* propagation of banana and plantain is that proposed by Murashige and Skoog (MS). The basal composition of salts and minerals in the MS formulation allows the explant to adapt to micropropagation processes, although many laboratories incorporate specific modifications based on the nutritional requirements of each plant (Galán et al., 2018). Other alternatives include the Gamborg (B5), Schenk and Hildebrandt (SH), and Linsmaier and Skoog (LS) media (Ngomuo et al., 2014). Therefore, the use of a culture medium with an appropriate composition is essential to ensure *in vitro* growth and development of each cultivar.

Plant regeneration can be achieved through organogenesis and somatic embryogenesis (Galán et al., 2018). The former is characterized by the formation of a unipolar primordium from a bud, giving rise to the development of a vegetative shoot that maintains a direct relationship with the maternal tissue. This technology is simple and well known and can be performed using buds, apices, or meristems; it has been widely used in systematic processes for commercial propagation. Somatic embryogenesis (SE), by contrast, consists of the formation of an embryo from a single cell or a group of cells. Somatic embryos do not have a vascular connection with the maternal tissue, as they are not products of gamete fusion; however, they must have the capacity to grow and form the entire plant structure. This method is considered the most efficient for *in vitro* plant propagation due to high multiplication rates in short periods of time and the ease of automating production processes (Morais-Lino et al., 2016). Nevertheless, its application in banana and plantain propagation is somewhat limited due to somaclonal variation and the scarcity of field studies on plants obtained through these processes.

4 Somatic embryogenesis

Somatic embryogenesis is a process that consists of embryo formation from a cell without the need for gamete fusion (Quiala et al., 2021). Cells undergo specific morphological and biochemical processes that lead to the formation of a somatic embryo (Shivani et al., 2017). Somatic embryos constitute new individuals and are characterized by a bipolar structure with apical and radical meristems, enabling them to develop into a complete plant (Horstman et al., 2017). SE represents a model of cellular totipotency involving signaling networks and reprogramming of gene expression patterns that are specifically regulated by plant growth regulators or environmental conditions (Nic-Can and Loyola-Vargas, 2016). It can occur via two pathways: direct and indirect (Grzyb et al., 2018). In direct SE, embryos at advanced developmental stages exhibit low uniformity and this approach is commonly used for plant regeneration. In contrast, indirect SE produces numerous somatic embryos at early stages with uniform development and is used to establish cell suspension cultures (Shivani et al., 2017). In monocotyledons, somatic embryo development progresses through globular, scutellar, and coleoptilar stages (Yuan et al., 2016).

The production of plants through SE consists of five phases: embryo induction, proliferation, maturation, germination, and plant conservation (Bradaï and Sánchez-Romero, 2021). Embryo induction begins with the formation of proembryogenic masses in culture media containing auxins to promote cellular aggregation. Subsequently, tissues are transferred to auxin-free media to stimulate cell division and embryo formation. Embryogenic tissues proliferate, leading to cell expansion and reserve accumulation; roots and shoots develop under *in vitro* conditions, and finally explants are transferred *ex vitro* for acclimatization and complete plant development (Quiala et al., 2021).

It is important to consider that donor tissues, culture media, and growth conditions influence plant regeneration via SE (Pencik et al., 2015). Therefore, understanding the physiological and molecular mechanisms involved in SE induction is essential (Méndez-Hernández et al., 2019). The addition of growth regulators in different proportions helps break dormancy and improves shoot forma-

tion (Márquez-López et al., 2018). Cytokinins are responsible for shoot formation and bud growth, whereas auxins are involved in root formation. SE employs biotechnological tools with the potential for genetic improvement and mass plant production (Zhou et al., 2016). Additionally, this technique is used in studies of cellular differentiation, gene expression, and molecular genetics. Embryogenic cell suspension cultures accelerate mass banana propagation due to their high regenerative potential and constitute an important non-conventional tool for plant improvement (Escobedo-Gracia et al., 2016).

5 Initiation of somatic embryogenesis in banana

The initiation of somatic embryogenesis is influenced by the selected plant material (Morais-Lino et al., 2016) and the balance of growth regulators in the culture medium (Tran et al., 2016). Uma et al. (2021) and Morais-Lino et al. (2016) report that the plant material used for SE in banana cultivation consists of male inflorescences, which should be collected during the tenth week after emergence (Natarajan et al., 2018) (Figure 3).

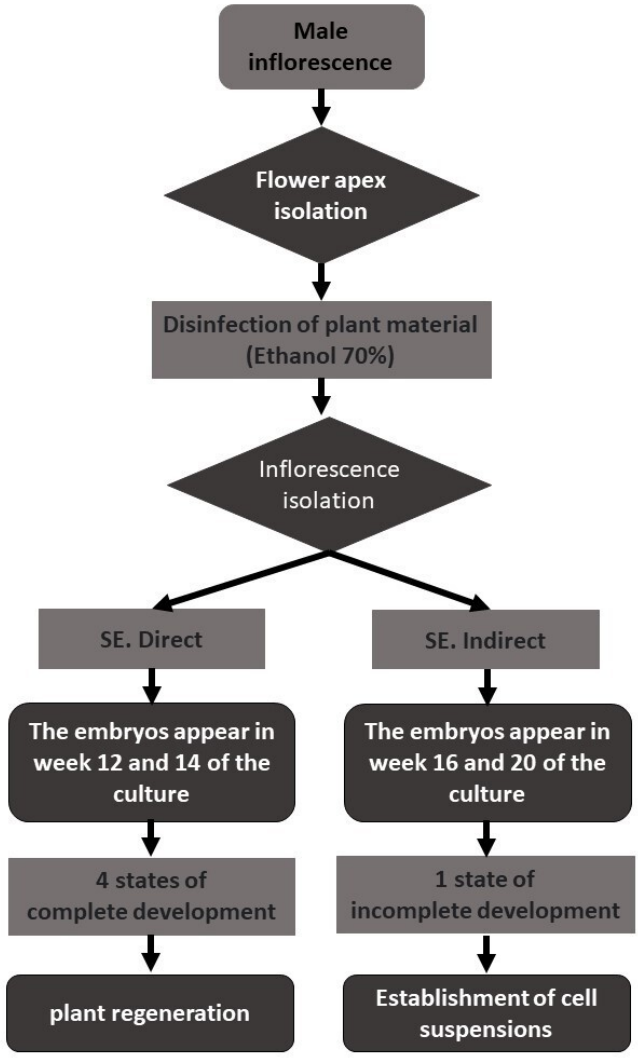

Figure 3. Process of somatic embryogenesis induction in banana.

Immature floral apices are sectioned layer by layer in a two-step process (Shivani et al., 2017). To remove bracts and prevent contamination by external agents, the material must be disinfected with 70% (v/v) ethanol to reduce exogenous contamination of plant tissues (Natarajan et al., 2018). Another key factor is the position of the hands obtained from the inflorescence; selection of the sixth to eighth hands allowed the formation of 50.0 ± 0.54% callus in 'Grand Naine' and 48.0 ± 1.67% in 'Rasthal'. According to Natarajan et al. (2018), this aspect influences embryogenic callus induction.

Floral apices are inoculated in MS medium containing salts, vitamins, 3% sucrose, and exogenous auxin-type growth regulators to induce the formation of cellular aggregates (Shivani et al., 2017). Morais-Lino et al. (2016) reported that growth regulator concentrations of 1 mg/L IAA + 4 mg/L 2,4-D + 1 mg/L NAA supplemented with glutamine induce callus formation in 50% of explants (Uma et al., 2021). In contrast, Youssef et al. (2010) reported that MS medium supplemented with 5.71 μM IAA, 18 μM 2,4-D, 5.4 μM NAA, 4.1 μM biotin, and 87 mM sucrose resulted in 81% callus formation in 'Williams' bud explants and 52.11% in 'Grand Naine' bud explants. Therefore, growth regulators enable the formation of embryogenic calli when applied at optimal concentrations according to cultivar and species. To induce SE, environmental factors must also be considered; thus, culture media containing plant material should be incubated at 27 °C under dark conditions for 12 days (Shivani et al., 2017) (Table 1).

Table 1. Composition of culture media for the initiation of indirect somatic embryogenesis in *Musa* spp.

Components	**Male flowers**	**Male flowers**	**Meristems**	**Male flowers**	**Male flowers**
Macroelements	MS	MS	MS	MS	MS
Microelements	MS	MS	MS	MS	MS
Vitamins	MS	MS	MS	MS	MS
Biotin	1 mg L^{-1}	4,1 μM	–	–	–
Malt extract	100 mg L^{-1}	–	–	–	–
NAA	1 mg L^{-1}	5,4 μM	–	1 mg L^{-1}	1 mg L^{-1}
IAA	1 mg L^{-1}	5,71 μM	–	1 mg L^{-1}	1 mg L^{-1}
2,4-D	4 mg L^{-1}	18 μM	–	4 mg L^{-1}	4 mg L^{-1}
Picloram	–	–	–	–	–
6-BA	–	–	13,31 μM	–	–
L-Glutamine	100 mg L^{-1}	–	–	–	50 mg L^{-1}
Sucrose	30 g L^{-1}	87 mM	30 g L^{-1}	3%	3%
Phytagel	2,6 g L^{-1}	–	–	–	–
Agar	–	–	7 g L^{-1}	0,70%	0,70%
Gelrite	–	2 g L^{-1}	–	–	–
pH	5,8	5,7	5,7	5,8	5,8
References	(S. Khalil et al., 2002)	(Youssef et al., 2010)	(Remakanthan et al., 2014)	(Morais-Lino et al., 2016)	(Morais-Lino et al., 2016)

6 Proliferation and initiation of embryogenic callus from cell suspension cultures

After the incubation period (4–6 months), the appearance of different types of calli begins. These are evaluated monthly during the first three months, followed by periodic evaluations every 15 days. After four months of culture, an embryogenic callus of friable nature develops, composed of whitish–translucent proembryogenic masses. Due to its characteristics, this callus is considered ideal for the establishment of cell suspension cultures (Morais-Lino et al., 2016).

Once the ideal calli have been identified, the establishment of embryogenic cell suspension cultures is initiated. According to Shivani et al. (2017), 24-week-old embryogenic calli are cultured in MS me-

La Granja: *Revista de Ciencias de la Vida* 43(1) 2026:128-144.

dium supplemented with 2,4-D, incubated at 27 °C in darkness, under constant agitation at 90 rpm, with subcultures performed every 7 days. In contrast, Strosse et al. (2003) report that calli should be transferred to a liquid MS medium supplemented with 1 mg/L IAA, 1.1 mg/L 2,4-D, and 250 μg/L zeatin, with the pH adjusted to 5.8, to promote the multiplication of embryogenic cells.

Table 2. Composition of culture media for the induction and proliferation of embryogenic calli

Components	cv. Dwarf Brazilian	M.a. cv. Grand Colla (AAA)	M.a. cv. Grand Naine (AAA)	M.a. cv. Grand Naine (AAA)
Macroelements	MS	MS 1/2	MS	MS
Microelements	MS	MS 1/2	MS	MS
Biotin (μM)	1 mg L^{-1}	–	–	–
Malt extract	100 mg L^{-1}	–	–	–
NAA	1 mg L^{-1}	–	–	–
IAA	1 mg L^{-1}	–	–	–
2,4-D	4 mg L^{-1}	4.5 μM	–	0.90 μM
Picloram	–	–	4.14 μM	–
6-BA	–	–	0.22 μM	–
L-Glutamine	100 mg L^{-1}	–	–	–
Sucrose	30 g L^{-1}	174 mM	30 g L^{-1}	30 g L^{-1}
Casein	200 mg L^{-1}	–	–	–
Proline	2 mg L^{-1}	–	–	–
Gelrite	–	2 g L^{-1}	–	–
Agar	–	–	7 g L^{-1}	7 g L^{-1}
KH_2PO_4	–	200 mg L^{-1}	–	–
pH	5.8	5.8	5.7	5.7
References	(S. Khalil et al., 2002)	(Youssef et al., 2010)	(Remakanthan et al., 2014)	(Remakanthan et al., 2014)

Alternatively, Morais-Lino et al. (2016) indicate that embryogenic masses are transferred to a liquid MS medium supplemented with 1 mg/L 2,4-D, 100 mg/L glutamine, 1 mg/L biotin, 10 mg/L ascorbic acid, and 44.5 g/L sucrose, under dark conditions at 27 $\pm$ 2 °C, with constant agitation at 120 rpm and subcultures every 10 days. At each subculture, the suspensions are filtered through a sieve to remove contaminated cells or cells that do not meet the characteristics required for the establishment of somatic embryogenesis from callus-derived embryogenic cell suspensions (ECS) (Morais-Lino et al., 2016).

One of the main problems in callus induction is the exudation of phenolic compounds into the culture medium, which are inherent to the nature of Musaceae and induce browning, decomposition, and hyperhydricity of the calli. An effective alternative to reduce phenolization levels is the use of antioxidants such as melatonin (50 mg L^{-1}) and L-glutamine (100 mg L^{-1}) in the callus induction medium (Natarajan et al., 2018).

Analysis of gene expression in embryogenic calli treated with 2,4-dichlorophenoxyacetic acid indicated that this growth regulator acts as an inducer of gene expression (*MaBBM1*, *MaBBM2*, *MaWUS2*, and *MaVP1*) (Shivani et al., 2017). This expression may be key to banana plant regeneration.

7 Development and maturation of the somatic embryo

Once the embryogenic callus has been formed (3–5 months) in a specific culture medium (Escobedo-Gracia et al., 2016), the next step is the proliferation of embryogenic tissue. This process is carried out in MS medium containing salts and vitamins, supplemented with 87 mM sucrose, 4.52 μM 2,4-D, 4.1 μM biotin, 680 μM glutamine, 100 mg L^{-1} malt extract, with the pH adjusted to 5.3, under constant agitation at 90 rpm and 27 °C in darkness. The medium must be renewed weekly, and the cell suspensions should be adjusted to a defined volume (Enríquez, 2019).

To determine SE growth and define the correct subculture times, it is necessary to establish the growth curve of the cell suspension. To do this, 1.5 ml of somatic cells are taken, the initial cell concentration is determined, and 50 ml of MS liquid medium is added. Growth is calculated by the change in cell volume for the evaluation interval every 5 days for 40 days. Likewise, the quality of embryogenic cell suspensions is determined by evaluating cell constitution (cell types), coloration, multiplication rate, and regeneration power (Morais-Lino et al., 2016).

Table 3. Comparison of the composition of culture media used for the initiation and maintenance of embryogenic cell suspension cultures

Components	**M.a. cv. Grand Naine (AAA)**	**M.a. cv. Grand Colla (AAA)**	**M. a. Colla (AA) ssp. Malaccensis**	**M.a. cv. Grand Naine (AAAAB) Tropical**
Macroelements	MS	MS	MS	MS
Microelements	MS	MS	MS	MS
Vitamins	MS	MS	MS	MS
Biotin	4,1 μM	–	4,1 μM	1 mg L^{-1}
2,4-D	4,5 μM	–	4,5 μM	1 mg L^{-1}
Picloram	–	–	2,1 μM	–
L-Glutamine	684 μM	–	684 μM	100 mg L^{-1}
Malt extract	100 mg L^{-1}	–	100 mg L^{-1}	1 mg L^{-1}
Sucrose	131 mM	87 mM	87 mM	44.5 g L^{-1}
Agar	–	–	–	–
pH	5,3	5,8	5,3	5,8
References	(Grapin et al., 2000)	(Youssef et al., 2010)	(Escobedo-Gracia et al., 2016)	(Morais-Lino et al., 2016)

8 Plants derived from somatic embryogenesis

The production of plants through the germination of somatic embryos with normal roots and shoots is achieved in substrates containing growth regulators (Escobedo-Gracia et al., 2014). Plant development depends on the genotype and on the procedures applied prior to and during embryo development (Morais-Lino et al., 2016). After the acclimatization and hardening process, conversion rates can be estimated, which are comparable to embryonic germination percentages. These percentages vary according to genotype, ranging from 3% to 46% in triploid Cavendish bananas (AAA); however, when somatic embryos are obtained from embryogenic cell suspension cultures, germination rates can reach up to 91% (Domergue et al., 2000). The highest rates were observed in triploid cv. Dwarf Brazilian (AAB) and *Musa acuminata* cv. Grand Naine (AAA), which share the characteristic that embryo development passes through a differentiation–maturation phase (Remakanthan et al., 2014). Somatic embryos obtained through somatic embryogenesis (SE) from shoot tips, as in the case of *Musa acuminata* AAA cv. Grand Naine, exhibit embryo conversion rates ranging from 2% to 3% (Remakanthan et al., 2014). Some SE protocols described for various banana genotypes report different percentages of somatic embryo germination and embryo conversion rates, since the data presented do not always clearly distinguish between these two processes (Escobedo-Gracia et al., 2016).

Conversion rates range from 13% in edible diploids (AA), from 13% to 25% in Grand Naine of the Cavendish subgroup (AAA), 66.7% in African Highland plantain (AAA) (Namanya et al., 2004), and up to 100% in wild *Musa acuminata* ssp. (AA) (Escobedo-Gracia et al., 2016). Regarding non-conventional improvement approaches (genetic transformation) aimed at counteracting pest problems and achieving higher germination rates, SE processes are essential components of *in vitro* regeneration systems, as they enable the development of resistant varieties (Ghag et al., 2014). For this reason, it is important to continue the ongoing development and optimization of SE protocols for the different cultivated clones (Escobedo-Gracia et al., 2016).

Table 4. Comparison of the composition of culture media used for the development and maturation of somatic embryos of *Musa* spp.

Components	**cv. Dwarf Brazilian (AAB)**	***M. a.* ssp. Malaccensis (AA)**	**cv. Grand Naine (AAA)**	***M.a.* cv. Grand Naine (AAA)**
Macroelements	MS	MS	MS	MS
Microelements	MS	MS	MS	MS
Vitamins	MS	MW	MS	MS
KH_2PO_4 (mM)	–	1.47 mM	–	–
Biotin	4.1 μM	–	–	–
Sucrose	131 mM	87.6 mM	30 g L^{-1}	30 g L^{-1}
BAP	–	–	2.2 μM	0.8 mg L^{-1}
NAA	5.4 μM	–	2.68 μM	–
Kinetin	2.3 μM	–	–	–
Zeatin	0.9 μM	–	–	–
Malt extract	100 mg L^{-1}	–	–	–
Glutamine	680 μM	–	–	–
Picloram	–	–	–	–
IAA	–	–	–	0.7 mg L^{-1}
Agar	–	–	–	7 g L^{-1}
Phytagel	2.6 g L^{-1}	–	4 g L^{-1}	–
Gelrite	–	2 g L^{-1}	–	–
pH	5.8	5.8	5.7	5.8
References	(S. M. Khalil and Elbanna, 2004)	(Escobedo-Gracia et al., 2016)	(Remakanthan et al., 2014)	(Morais-Lino et al., 2016)

Table 5. Culture medium composition commonly used for the germination of somatic embryos in *Musa* spp.

Components	**cv. Grand Naine (AAA)**	***M.a.* cv. Grand Colla (AAA)**	***M.a.* cv. Grand Naine (AAA)**	***M.a.* cv. Grand Naine (AAA)**	**Tropical (AAAAB)**
Macroelements	MS	MS	MS	MS	MS
Microelements	MS	MS	MS	MS	MS
Vitamins	MS	MS	MS	MS	MS
KH_2PO_4 (mM)	1,47 mM	–	–	–	–
Sucrose	–	87 mM	–	30 g L^{-1}	30 g L^{-1}
BAP	–	0,22 μM	0,22 μM	0,8 mg L^{-1}	0,2 mg L^{-1}
Picloram	–	–	4,14 μM	–	–
IAA	1,14 μM	1,14 μM	–	0,7 mg L^{-1}	0,1 mg L^{-1}
Agar	–	–	–	7 g L^{-1}	7 g L^{-1}
Gelrite	–	2 g L^{-1}	–	–	–
pH	–	5,8	–	–	–
Number of embryos	–	–	355,83±31,72	6076,6	106,0
% Germination	35–46	35	100	–	–
% Conversion	–	–	–	20,21	79,72
References	(S. M. Khalil and Elbanna, 2004)	(Youssef et al., 2010)	(Remakanthan et al., 2014)	(Morais-Lino et al., 2016)	(Morais-Lino et al., 2016)

Table 6. Biotechnological companies with phytosanitary certification to import *in vitro* banana plants (*Musa sapientum*) into Ecuador.

Company	Country of origin	Phytosanitary Certificate Plants free of:
Agribiotecnología / Cristal Vitro	Costa Rica	Banana bunchy top virus (BBTV) Banana streak badnavirus (BSV) Banana bract mosaic potyvirus (BBMV) Banana bract mosaic virus (BBrMV) Abaca mosaic virus (ABTV) Abaca mosaic virus (AbaMV) Banana mild mosaic virus (BanMMV) Banana virus X (BVX) *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4
Genética Salvadoreña S.A.	El Salvador	Cucumber mosaic virus (CMV) Abaca mosaic virus (ABTV) Banana bunchy top virus (BBTV) Banana streak badnavirus (BSV) Banana bract mosaic virus (BBMV) Banana mild mosaic virus (BanMMV) Banana virus X (BVX) y *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4
Galiltec S.A.	Honduras	Banana streak virus (BSV) Banana bunchy top virus (BBTV) Banana bract mosaic potyvirus (BBMV) Banana bract mosaic virus (BBrMV) Abaca mosaic virus (ABTV) Abaca mosaic virus (AbaMV) Banana mild mosaic virus (BanMMV) Banana virus X (BVX) *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4
Rahan Meristem del Ecuador Cía. Ltda	Israel	Banana bunchy top virus (BBTV), Cucumber mosaic virus (CMV) Banana streak badnavirus (BSV), Banana bract mosaic virus (BBrMV) Abaca bunchy top virus (ABTV) Abaca mosaic virus (AbaMV) Banana mild mosaic virus (BanMMV) Banana virus X (BVX) *Fusarium oxysporum* f. sp. *cubense* Raza 4 Tropical
Nature Source Improves Plants de México, S.A.	México	Banana bunchy top virus (BBTV) Banana streak badnavirus (BSV) Banana bract mosaic potyvirus (BBMV) Banana bract mosaic virus (BBrMV) Abaca mosaic virus (ABTV) Abaca mosaic virus (AbaMV) Banana mild mosaic virus (BanMMV) Banana virus X (BVX) *Fusarium oxysporum* f. sp. *cubense* Tropical Race 4
Du Roi	Sudáfrica	Banana bunchy top virus (BBTV) Banana rayado badnavirus (BSV) Banana bract mosaic potyvirus (BBMV) Banana bract mosaic virus (BBrMV) Abaca mosaic virus (ABTV) Abaca mosaic virus (AbaMV) Banana mild mosaic virus (BanMMV) *Fusarium oxysporum* f. sp. *cubense* Raza 4 Tropical

Source: Agrocalidad, (2022)

Table 7. Registered establishments for the *in vitro* propagation of banana plant material (*Musa sapientum*).

Legal Name	Canton	Status	Type of Operation	Type/Area
BIOFABRICA MONTUBIA S.A.S.	Machala	Registered	Meristematic micropropagator	Micropropagation Laboratory
SEBIOCA C.A. EN LIQUIDACIÓN	Guayaquil	Registered	Meristematic micropropagator	Micropropagation Laboratory
RAHAN MERISTEM DEL ECUADOR CIA. LTDA.	Guayaquil	Registered	Meristematic micropropagator	Micropropagation Laboratory
VITROLIFE S.A.	Guayaquil	Registered	Meristematic micropropagator	Micropropagation Laboratory
ONE VITRO S.A.	Durán	Registered	Meristematic micropropagator	Micropropagation Laboratory
BIOGENETICAGREEN C.A.	Quito	Registered	Meristematic micropropagator	Micropropagation Laboratory
GERMOPLANTA CIA. LTDA.	Rumiñahui	Registered	Meristematic micropropagator	Micropropagation Laboratory
ORANGELAB S.A.	Quito	Registered	Meristematic micropropagator	Micropropagation Laboratory
YURA BUSINESS S.A.S.	Quito	Registered	Meristematic micropropagator	Micropropagation Laboratory

Source: Agrocalidad, (2022)

9 Somaclonal variation in plants regenerated through somatic embryogenesis

A feature of *in vitro* plant tissue cultures is the occurrence of somaclonal variation, involving genetic changes in cultured cells and tissues (Nwauzoma and Jaja, 2013). In some cases, this variation is exploited for genetic improvement, allowing the expansion of natural genetic variability (Wang et al., 2021); however, when clonal propagation is intended, somaclonal variation represents an undesirable anomaly.

The *in vitro* culture environment, including the type and concentration of plant growth regulators, together with the genetic background of the explant, as well as the total number and duration of subcultures, can alter the characteristics of plants regenerated from somatic embryos. All these factors contribute to the generation of genetic and epigenetic variation (Escobedo-Gracia et al., 2014), which is expressed at the phenotypic level and is known as somaclonal variation (Youssef et al., 2010). This variation may constitute pre-existing genetic variation in the explant due to changes in chromosome number or variation induced by *in vitro* culture conditions; additionally, DNA mutations and epigenetic changes at the sequence level may also occur (Wang et al., 2021).

It is also known that somaclonal variation in banana cultivation is associated with long-term cultures or cultures that involve a callus phase or high rates of multiplication treatments (Nwauzoma and Jaja, 2013). On the other hand, the reduction in the regeneration capacity of cultures derived from embryogenic cell suspensions is associated with cytogenetic instability in triploid bananas (AAA genome) of the Cavendish subgroup, off-type regenerants from long-term cell suspension cultures, and the subsequent loss of regeneration potential (Wu et al., 2020).

The regeneration process via somatic embryogenesis showed greater genetic stability compared with plantlets regenerated from long-term cultures, in which a higher DNA content was detected (Escobedo-Gracia et al., 2016). Regarding genetic instability/stability, when evaluating morphological and agronomic parameters, variation ranges from approximately 0.3% to 3.6%; meanwhile, molecular markers recorded low variation levels between 1.4% and 1.6%, within the natural variation found in the mother plant used as the explant source (Ghag et al., 2014).

Table 8. Somaclonal variation in banana plants regenerated through *in vitro* somatic embryogenesis.

Plant material (genome composition)	**Tissue /source of variation**	**Detection method**	**Percentage of variation (%)**	**References**
M. a. (AAA) cv. Grand Naine	Embryogenic culture	AFLP	1.4	Youssef et al. (2010)
M. a. (AAA) cv. Williams	Embryogenic culture	AFLP	1.6	Youssef et al. (2010)
Grand Naine (AAA)	Cell suspensions	SSR primers	0	Morais-Lino et al. (2016)
Tropical (AAAB)	Cell suspensions	SSR primers	0	Morais-Lino et al. (2016)
cv. Valery (AAA)	Embryogenic culture	Chromosome counting	30 % aneuploidy	Moradi et al. (2017)

Table 9. Results of banana genetic improvement through *in vitro* plant generation processes.

Cultivar	**Tissue / origin**	**Expressed gene / target gene**	**Gene transfer technique**	**Modified trait**	**Transgenic plant**	**References**
Grand Nain (AAA)	ECS (male flowers)	Endochitinase gene *ThEn-42* from *Trichoderma* cwith grape stilbene synthase gene (StSy) and tomato (Cu, Zn-SOD) superoxide dismutase gene	Biolistics	Resistance to *M. fijiensis*	Yes	Vishnevetsky et al. (2011)
Gros Michel (AAA)	ECS (male flowers)	Rice chitinase genes	*Agrobacterium*	Resistance to *M. fijiensis*	Yes	Kovács et al. (2013)
Musa acuminata L. AA, cv. Mas	Male flowers – direct organogenesis	GUS	Ballistics + *Agrobacterium*	–	Yes	Liu et al. (2017)
Cavendish "Williams"	ECS (male flowers)	gRNA	CRISPR/ Cas9	Disease resistance	Yes	Naim et al. (2018)
"Gonja manjaya" (AAB)	*in vitro* plants	gRNA1 (ORF1), gRNA2 (ORF2), gRNA3 (ORF3)	CRISPR/ Cas9	Resistance to banana streak virus (BSV)	Yes	Tripathi et al. (2019)
Cavendish (AAA)	Protoplasts	sgRNA	CRISPR/ Cas9	Disease resistance	Yes	Wu et al. (2020)

Note: ECS = Embryogenic Cell Suspension .

10 Genetic transformation of banana using somatic embryogenesis cultures

The development of improved banana varieties through conventional methods represents a challenge due to low genetic variability, polyploidy, and sterility of commercial cultivars (Tripathi et al., 2019). Therefore, somatic embryogenesis constitutes an alternative technique for banana plant improvement (Liu et al., 2017). Likewise, the use of protoplasts obtained through embryogenesis from embryogenic cell suspension cultures (ECS) is favored because of their high yield, high activity, ease of operation, and broad adaptability (Wu et al., 2020). Genetic transformation methods used in banana include *Agrobacterium*-mediated transformation (Kovács et al., 2013), particle bombardment (Vishnevetsky et al., 2011), and CRISPR/Cas9 (Wu et al., 2020).

Genome-editing technologies are valuable tools for exploring the underlying mechanisms of gene function and regulation and can serve as a platform for crop genetic improvement through the removal of undesirable chromosomal DNA, positive or negative regulation of endogenous genes, and the introduction of novel coding sequences (Liu et al., 2017). Table 9 describes the main results obtained in banana plant regeneration.

11 Conclusions

Banana propagation through somatic embryogenesis represents an alternative for plant multiplication due to its high potential for tissue regeneration within a short period of time. However, the risk of somaclonal variation that explants may exhibit has limited its expansion at the commercial scale. Therefore, it is necessary to develop specific protocols for each species, select appropriate plant material, and identify the type of genotype to ensure the success of plant micropropagation. On the other hand, the genetic variability exhibited by explants subjected to this technique has been used in studies related to the genetic transformation of banana plants.

The susceptibility of the crop to diseases such as *M. fijiensis*, *Fusarium oxysporum* f. sp. *cubense* (Foc TR4), and banana streak virus (BSV) has encouraged the development of new *in vitro* establishment protocols for resistant species. Large-scale propagation of plants through embryogenesis is an effective alternative for banana monocultures during each renewal cycle; therefore, process traceability is necessary to identify mutations. The application of embryogenic cell suspensions or the combined use of protoplasts with gene transfer or genome-editing techniques has enabled the development of new plant types with resistance or tolerance to the main diseases affecting the crop.

Author Contributions

J.R.L.M.: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Software, Validation, Writing – original draft, Writing – review & editing. **J.W.M.T.:** Funding acquisition, Supervision. **I.C.L.M.:** Validation, Project administration. **Y.A.O.G.:** Resources, Visualization.

References

Ahmed, S., Sharma, A., Singh, A. K., Wali, V. K., and Kumari, P. (2014). In vitro multiplication of banana (*Musa* sp.) cv. Grand Naine. *African Journal of Biotechnology*, *13*(27). Online: https://doi.org/10.5897/AJB2014.13750

Anbazhagan, M., Balachandran, B., and Arumugam, K. (2014). In vitro propagation of *Musa* sp. (banana). *International Journal of Current Microbiology and Applied Sciences*, *3*(7), 399–404. Online: https://tinyurl.com/23lh8gk9

Bradaï, F., and Sánchez-Romero, C. (2021). Effect of Cryopreservation on the Ex-Vitro Establishment of Olive Plants Regenerated via Somatic Embryogenesis. *Plants*, *10*(2), 2223–7747. Online: https://doi.org/10.3390/plants10020396

Capa Benítez, L. B., Alaña Castillo, T. P., and Benítez Narváez, R. M. (2016). Importancia de la producción de banano orgánico: Caso provincia El Oro, Ecuador. *Revista Universidad y Sociedad*, *8*(3), 64–71. Online: https://tinyurl.com/24w794kz

Domergue, F. G. R., Ferrière, N., and Côte, F. X. (2000). Morphohistological study of the different

constituents of a banana (*Musa* AAA, cv. Grande naine) embryogenic cell suspension. *Plant Cell Reports*, *19*, 748–754. Online: https://doi.org/10.1007/s002999900188

Enríquez, A. (2019). *Análisis fisiológico, bioquímico y molecular del desarrollo y maduración del embrión somático* (Tesis Doctoral, Tesis doctoral). Online: https://tinyurl.com/yxoqejyq

Escobedo-Gracia, R. M., Enríquez-Valencia, A. J., Youssef, M., López-Gómez, P., Cruz-Cárdenas, C. I., and Ku-Cauich, J. R. (2016). Somatic Embryogenesis in Banana, *Musa* ssp. En V. Loyola-Vargas and N. Ochoa-Alejo (Eds.), *Somatic Embryogenesis: Fundamental Aspects and Applications.* Cham: Springer. Online: https://doi.org/10.1007/978-3-319-33705-0_21

Escobedo-Gracia, R. M., Maldonado-Borges, J. I., Burgos-Tan, M. J., Valadez-González, N., and Ku-Cauich, J. R. (2014). Using flow cytometry and cytological analyses to assess the genetic stability of somatic embryo-derived plantlets from embryogenic *Musa acuminata* Colla (AA) ssp. *malaccensis* cell suspension cultures. *Plant Cell, Tissue and Organ Culture (PCTOC)*, *116*, 175–185. Online: https://doi.org/10.1007/s11240-013-0394-z

FAO. (2020). *Examen del mercado del banano: resultados preliminares de 2019* (Inf. Téc.). Roma: Food and Agriculture Organization.

Galán, V., Rangel, A., López, J., Hernández, J. B. P., Sandoval, J., and Rocha, H. S. (2018). Propagación del banano: técnicas tradicionales, nuevas tecnologías e innovaciones. *Revista Brasileira de Fruticultura*, *40*(4). Online: https://doi.org/10.1590/0100-29452018574

Ghag, S., Shekhawat, U. S., and Ganapathi, T. (2014). Transgenic banana plants expressing a *Stellaria media* defensin gene (Sm-AMP-D1) demonstrate improved resistance to *Fusarium oxysporum*. *Plant Cell, Tissue and Organ Culture (PCTOC)*, *119*, 247–255. Online: https://doi.org/10.1007/s11240-014-0529-x

Grapin, A., Ortiz, J. L., Domergue, R., Babeau, J., Monmarson, S., Escalant, J. V., … Côte, F. (2000). Establishment of embryogenic callus and initiation and regeneration of embryogenic cell suspensions from female and male immature flowers of *Musa*. *Musalit*, *7*(1), 13–15. Online: https://www.musalit.org/seeMore.php?id=13956

Grzyb, M., Kalandyk, A., and Mikula, A. (2018). Efecto de TIBA, fluridona y ácido salicílico sobre la embriogénesis somática y el contenido de hormonas y azúcares endógenos en el helecho arborescente *Cyathea delgadii* Sternb. *Acta Physiologiae Plantarum*, *40*, 1. Online: https://doi.org/10.1007/s11738-017-2577-4

Horstman, A., Bemer, M., and Boutilier, K. (2017). Una visión transcripcional de la embriogénesis somática. *Regeneración*, *4*(4), 201–216. Online: https://doi.org/10.1002/reg2.91

Khalil, S., Cheah, K., Perez, E., Gaskill, D., and Hu, J. (2002). Regeneration of banana (*Musa* spp. AAB cv. Dwarf Brazilian) via secondary somatic embryogenesis. *Plant Cell Reports*, *20*(12), 1128–1134. Online: https://doi.org/10.1007/s00299-002-0461-0

Khalil, S. M., and Elbanna, A. A. M. (2004). Highly efficient somatic embryogenesis and plant regeneration via suspension cultures of banana (*Musa* ssp.). *Arab Journal of Biotechnology*, *7*, 99–110.

Kovács, G., Sági, L., and Jacon, G. (2013). Expression of a rice chitinase gene in transgenic banana ('Gros Michel', AAA genome group) confers resistance to black leaf streak disease. *Transgenic Research*, *22*, 117–130. Online: https://doi.org/10.1007/s11248-012-9631-1

Liu, J., Gao, P., Sun, X., Zhang, J., Sun, P., Wang, J., … Jin, Z. (2017). Efficient regeneration and genetic transformation platform applicable to five *Musa* varieties. *Electronic Journal of Biotechnology*, *25*, 33–38. Online: https://doi.org/10.1016/j.ejbt.2016.11.002

Lohidas, J., and Sujin, D. (2015). Effect of growth hormones in the micro propagation of Banana cv. Matti. *Plant Archives*, *15*(1), 307–314. Online: https://tinyurl.com/29hd3t9e

MAGAP. (2016). *Análisis Sectorial BANANA* (Inf. Téc.). Quito: Pro Ecuador. Online: https://tinyurl.com/y6ywd4pt

Martin, G., Cardi, C., Sarah, G., Ricci, S., Jenny, C., Fondi, E., … Yahiaoui, N. (2020). Genome ancestry mosaics reveal multiple and cryptic con-

tributors to cultivated banana. *The Plant Journal*, *102*(5), 1008–1025. Online: https://doi.org/10.1111/tpj.1468

Moradi, Z., Farahani, F., Sheidai, M., and Nejad Satari, T. (2017). Somaclonal variation in banana (*Musa acuminata* colla cv. Valery) regenerated plantlets from somatic embryogenesis: histological and cytogenetic approaches. *Caryologia*, *70*(1), 1–6. Online: https://doi.org/10.1080/00087114.2016.1198665

Morais-Lino, L. S., Santos-Serejo, J. A., Amorim, E. P., de Santana, J. R. F., Pasqual, M., and de Oliveira e Silva, S. (2016). Somatic embryogenesis, cell suspension, and genetic stability of banana cultivars. *In Vitro Cellular & Developmental Biology-Plant*, *52*(1), 99–106. Online: https://doi.org/10.1007/s11627-015-9729-2

Moreno, B., Muñoz, M., Cuellar, J., Domancic, S., and Villanueva, J. (2018). Revisiones sistemáticas: definición y nociones básicas. *Revista clínica de periodoncia, implantología y rehabilitación oral*, *11*(3), 184–186.

Márquez-López, R. E., Pérez-Hernández, C., Ku-González, , Galaz-Ávalos, R. M., and Loyola-Vargas, V. M. (2018). Localization and transport of indole-3-acetic acid during somatic embryogenesis in *Coffea canephora*. *Protoplasma*, *255*(2), 695–708. Online: https://tinyurl.com/y2ugj6mf

Méndez-Hernández, H., Ledezma-Rodríguez, M., Avilez-Montalvo, R., Juárez-Gómez, Y., Skeete, A., Avilez-Montalvo, J., … Loyola-Vargas, V. (2019). Signaling overview of plant somatic embryogenesis. *Frontiers in Plant Science*, *10*, 77. Online: https://doi.org/10.3389/fpls.2019.00077

Naim, F., Dugdale, B., Kleidon, J., Brinin, A., Shand, K., Waterhouse, P., and Dale, J. (2018). Gene editing the phytoene desaturase alleles of Cavendish banana using CRISPR/Cas9. *Transgenic Research*, *27*(5), 451–460. Online: https://link.springer.com/article/10.1007/s11248-018-0083-0

Namanya, P., Magambo, S. M., Mutumba, G., and Tushemereirwe, W. (2004). Somatic embryogenesis from immature male inflorescences of East African highland banana CV 'Nakyetengu'. *African Crop Science Journal*, *12*(1), 43–49. Online: https://tinyurl.com/y2bknwou

Natarajan, N., Krish, K., Duraialagaraja, S., and Soorianathasundaram, K. (2018). Plant regeneration, developmental pattern and genetic fidelity of somatic embryogenesis derived *Musa* spp. *Journal of Genetic Engineering and Biotechnology*, *16*(2), 587–598. Online: https://doi.org/10.1016/j.jgeb.2018.10.001

Ngomuo, M., Mneney, E., and Ndakidemi, P. A. (2014). The in vitro propagation techniques for producing banana using shoot tip cultures. *American Journal of Plant Sciences*. Online: https://doi.org/10.4236/ajps.2014.511175

Nic-Can, G. I., and Loyola-Vargas, V. M. (2016). El papel de las auxinas durante la embriogénesis somática. En V. M. Loyola-Vargas and N. Ochoa-Alejo (Eds.), *Embriogénesis somática. Aspectos fundamentales y aplicaciones* (pp. 171–181). Cham: Springer. Online: https://doi.org/10.1007/978-3-319-33705-0_10

Nwauzoma, A. B., and Jaja, E. T. (2013). A review of somaclonal variation in plantain (*Musa* spp): mechanisms and applications. *Journal of Applied Biosciences*, *67*, 5252–5260. Online: https://doi.org/10.4314/jab.v67i0.95046

Pencik, A., Tureková, V., Paulisiç, S., Rolcìk, J., Strnad, M., and Mihaljevic, S. (2015). El amonio regula el potencial embriogénico en *Cucurbita pepo* a través de cambios mediados por el pH en la auxina endógena y el ácido abscísico. *Cultivo de órganos de tejido celular vegetal*, *122*, 89–100. Online: https://doi.org/10.1007/s11240-015-0752-0

Quiala, E., Barbón, R., Mestanza, S., La O, M., Gardenis, M., Nuñez, J., … Noceda, C. (2021). Somatic embryogenesis and plant regeneration from leaf of the interspecific hybrid of mahogany (*Swietenia macrophylla* King × *S. mahagoni* (L.) Jacq.). *Trees*. Online: https://doi.org/10.1007/s00468-021-02192-x

Remakanthan, A., Menon, T. G., and Soniya, E. V. (2014). Somatic embryogenesis in banana (*Musa acuminata* AAA cv. Grand Naine): effect of explant and culture conditions. *In Vitro Cellular & Developmental Biology - Plant*, *50*(1), 127–136. Online: https://doi.org/10.1007/s11627-013-9546-4

Rodríguez, P. A. (2013). Cultivo de tejidos [Manual de software informático]. Bogotá, Colombia.

Online: https://tinyurl.com/y5cuv8z6 (Módulo Escuela de Ciencias Agrícolas Pecuarias y del Medio Ambiente)

Shivani, Awasthi, P., Sharma, V., Kaur, N., Kaur, N., Pandey, P., and Tiwari, S. (2017). Genome-wide analysis of transcription factors during somatic embryogenesis in banana (*Musa* spp.) cv. Grand Naine. *PLoS ONE*, *12*(8), e0182242. Online: https://doi.org/10.1371/journal.pone.0182242

Simoníková, D., Cízková, J., Zoulová, V., Christelová, P., and Hribová, E. (2022). Advances in the Molecular Cytogenetics of Bananas, Family Musaceae. *Plants*, *11*(4), 482. Online: https://doi.org/10.3390/plants11040482

Strosse, H., Domergue, R., Panis, B., Escalant, J. V., and Côte, F. (2003). *Banana and plantain embryogenic cell suspensions* (Technical Guidelines 8). Montpellier, France: The International Network for the Improvement of Banana and Plantain (INIBAP).

Taco, J., Jiménez, R., and Soto, M. (2026). Valorization of banana peel (musa paradisiaca) as a raw material for biopolymer production. *La Granja: Revista de Ciencias de la Vida*, *43*(1), 158–169. Online: https://doi.org/10.17163/lgr.n43.2026.09

Tran, T. H., Bui, T. V., and Feng, T. Y. (2016). The role of auxin and cytokinin on somatic embryogenesis from cell suspension cultures of the banana cultivar "Cau Man". *Acta Horticulturae*(1114), 219–226. Online: https://doi.org/10.17660/actahortic.2016.1114.30

Tripathi, J. N., Ntui, V. O., Ron, M., Muiruri, S. K., Britt, A., and Tripathi, L. (2019). CRISPR/Cas9 editing of endogenous banana streak virus in the B genome of *Musa* spp. overcomes a major challenge in banana breeding. *Communications Biology*, *2*(1), 1–11. Online: https://www.nature.com/articles/s42003-019-0288-7

Uma, S., Kumaravel, M., Backiyarani, S., Saraswathi, M., Durai, P., and Karthic, R. (2021). La embriogénesis somática como herramienta para la reproducción de plantas genéticamente estables en banano y ensayos de campo confirmatorios. *Plant Cell, Tissue and Organ Culture (PCTOC)*, *147*, 181–188. Online: https://doi.org/10.1007/s11240-021-02108-0

Vishnevetsky, J., White, T. L., Palmateer, A. J., Flaishman, M., Cohen, Y., Elad, Y., … Perl, A. (2011). Improved tolerance toward fungal diseases in transgenic Cavendish banana (*Musa* spp. AAA group) cv. Grand Nain. *Transgenic Research*, *20*(1), 61–72. Online: https://tinyurl.com/y6sjbjed

Wang, X., Yu, R., and Li, J. (2021). Using genetic engineering techniques to develop banana cultivars with fusarium wilt resistance and ideal plant architecture. *Frontiers in Plant Science*, *11*, 617528. Online: https://doi.org/10.3389/fpls.2020.617528

Wu, S., Zhu, H., Liu, J., Yang, Q., Shao, X., Bi, F., … Yi, G. (2020). Establishment of a PEG-mediated protoplast transformation system based on DNA and CRISPR/Cas9 ribonucleoprotein complexes for banana. *BMC Plant Biology*, *20*(1), 425. Online: https://doi.org/10.1186/s12870-020-02609-8

Youssef, M., James, A., Mayo-Mosqueda, A., Ku-Cauich, J., Grijalva, R., Escobedo, R., and Medrano, G. (2010). Influence of genotype and age of explant source on the capacity for somatic embryogenesis of two Cavendish banana cultivars (*Musa acuminata* Colla, AAA). *African Journal of Biotechnology*, *9*(15), 2216–2223. Online: https://doi.org/10.4314/ajb.v9i15

Yuan, G., Shui-Gen, L., Xiao-Fen, F., and Zhen-Hong, S. (2016). Application of Somatic Embryogenesis in Woody Plants. *Frontiers in Plant Science*, *7*, 938. Online: https://doi.org/10.3389/fpls.2016.00938

Zhou, Q., Wang, M., Yuan, Y., Wang, X., Fu, R., Wan, H., … Zhou, Q. (2016). Complete meiosis from embryonic stem cell-derived germ cells in vitro. *Cell Stem Cell*, *18*(3), 330–340. Online: https://doi.org/10.1016/j.stem.2016.01.017