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Somatic embryogenesis and *in vitro* plant regeneration of Manzano (AAB) and Pelipita (ABB) banana cultivars

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Abstract: There is significant interest in the propagation of bananas due to their substantial global economic importance, which is why this study aimed to develop an efficient protocol for *in vitro* propagation through somatic embryogenesis (SE) of the 'Manzano' and 'Pelipita' cultivars. Immature male flower bud explants were used for embryogenic callus induction. The 'Manzano' cultivar showed a higher percentage of embryogenic callus induction than 'Pelipita'. Stereomicroscopic observations revealed that the embryogenic callus was asynchronous, as various stages of somatic embryos were noted. White translucent pro-embryogenic callus was efficiently utilized to establish embryogenic cell suspension. The development of different stages in the regeneration process of embryogenic cell suspension was also recorded. Embryos in the late coleoptilar stage, characterized by an opaque white morphology, were selected and transferred to an appropriate medium to evaluate the germination percentage. We found that the germination rate was highly efficient for both cultivars, exceeding 90% when using these embryos in the selected stage. Additionally, we observed that embryos that were 90 days old showed a 10–12% increase in germination compared to those that were 60 days old, confirming the importance of embryo maturation duration and the selection of embryogenic aggregates through the sieving of suspended cells to optimize regeneration efficiency.

Keywords: asexual propagation; bananas and plantains; cell suspension cultures; plant micropropagation

The genus *Musa* comprises bananas and plantains; they are evergreen crops and are among the most important tropical and subtropical fruits worldwide. Their importance stems from factors ranging from nutritional value to economic impact in developing countries (Escobedo-GraciaMedrano et al. 2016; Yadav 2021). In many regions, this fruit is a staple food and contributes to a balanced diet as a source of carbohydrates, vitamins, minerals, and fibre (Kumari et al. 2023). In 2022 alone, 179 million tons (Mtons) of this fruit were produced, with India being the leading producer,

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followed by China and Uganda (FAOSTAT 2024). In Mexico, over the last five years, production reached 12.2 Mtons, averaging 2.4 Mtons per year.

The *Musa* genus belongs to the Musaceae family (Zingiberales order), and five distinct sections of the genus were once recognised; however, based on seed morphology, molecular data, chromosome numbers, and even male floral morphology, these sections have recently been merged into two sections: Callimusa and Musa (Häkkinen 2013; Inta et al. 2023). The latter is composed of the two wild diploid species *Musa acuminata* (AA) and *M. balbisiana* (BB), which, through inter/intraspecific crossing, have given rise to the commercial cultivars we know today (Wang et al. 2019).

Eight varieties of bananas and plantains in Mexico are primarily marketed for commercial purposes: 'Giant Dwarf', 'Pear', 'Dominican', 'Tabasco', 'Valery', 'Macho', 'Purple', and 'Manzano' plantains. These cultivars exhibit considerable diversity in flavour and texture, catering to a broad range of consumer preferences. However, in recent years, the production of traditional, locally cultivated varieties, such as Manzano, has significantly decreased in the market. This decline is attributed to the widespread commercialisation of agronomically focused cultivars and the impact of biotic factors, including pests and diseases (FAOSTAT 2024). Consequently, promoting the diversification of commercially viable cultivars and introducing varieties with desirable agronomic and sensory characteristics is essential. In this context, the 'Pelipita' cultivar represents a valuable alternative, combining favourable sensory attributes with resistance to various pests and diseases, offering potential benefits for producers and consumers (García-Velasco et al. 2020). Therefore, the diversification of banana cultivars is of considerable importance, as it contributes to food security, supports local consumption, and promotes the genetic conservation of the *Musa* species.

Commercial cultivars have a reduced genetic heritage; they are primarily triploid and develop fruits without fertilisation (parthenocarpy). Due to their poor fertility, plants are propagated asexually mainly through offshoots, which are shoots born from the main rhizome and can give rise to new plants that are genetically identical to the original. As a result, the crop is threatened by various pests and diseases caused by viruses, bacteria, fungi, and insects. Among the diseases that significantly impact the crop are black leaf streak disease, known as black sigatoka (caused by *Pseudocercospora fijiensis*) (Yonow et al. 2019), yellow Sigatoka (*Pseudocercospora musicola*) (Drenth, Kema 2021), banana bunchy top virus (BBTV) (Qazi 2016), Xanthomonas wilt (*Xanthomonas campestris* pv. *musacearum*) (Tinzaara et al. 2024), and Fusarium wilt (caused by *Fusarium oxysporum* f.sp. *cubense* (*Foc*)) (Ploetz 2015). The latter is the most devastating disease for banana and plantain crops worldwide. *Foc* has caused significant economic losses due to its high crop dispersion and long soil survival, leading to its classification as a quarantine pest worldwide (Olivares et al. 2021; EFSA PLH Panel et al. 2022). In Mexico, the disease caused by *Foc* was first reported in 1932 in the states of Michoacán and Puebla. The introduction of *Fusarium oxysporum* f.sp. *cubense* race 1 (*Foc*R1) resulted in the loss of approximately 40 000 hectares of the 'Cavendish' cultivar. Subsequently, in 1994, an additional 4 000 hectares of the 'Manzano' cultivar were affected, leading to considerable economic losses for small-scale producers (Florencio Anastasio et al. 2023). Notably, because of its characteristics, such as sterility and polyploidy, genetic transformation and gene editing have been considered promising alternatives since improving specific traits is not always possible through recurrent selection breeding programs (Ganapathi et al. 2021; Justine et al. 2022; Tripathi et al. 2024).

As an example, we refer to the efforts of the Honduran Foundation for Agricultural Research (FHIA), which, despite obtaining some disease-resistant tetraploid cultivars, have mostly been poorly accepted due to their organoleptic properties (Rowe, Rosales 1994; Aguilar Morán 2011). Additional efforts include biotechnological approaches developed to improve this species, such as genetic improvement assisted by molecular markers (Crouch et al. 2000), protoplast fusion (Assani et al. 2005), genetic transformation by particle bombardment (Sági et al. 1995; Becker et al. 2000), and, as mentioned before, *Agrobacterium tumefaciens*-mediated genetic transformation (Subramanyam et al. 2011; Ganapathi et al. 2021) and/or gene editing (Tripathi et al. 2024). Note that these latter approaches could be limited by the low rates of *in vitro* regeneration. Therefore, efficient propagation methods, such as regeneration via somatic embryogenesis, are required.

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In plants, SE is the process through which somatic cells differentiate into bipolar somatic embryos, capable of regenerating a whole plant without a fertilisation event (Zimmerman 1993; Méndez-Hernández et al. 2019). This tool has been applied in various crops such as *Daucus carota* (Kamada et al. 1989), *Coffea canephora* (Etienne 2005), *C. arabica* (Molina et al. 2002), *Zea mays* (Lu et al. 1982), and *Musa acuminata* (Jalil et al. 2003). During somatic embryogenesis, several transition stages for somatic cells have been described, including somatic cell induction, proliferation of embryogenic callus, maturation of somatic embryos, and germination (Adero et al. 2023). The advantages of this system include a high multiplication rate for obtaining seedlings in a short time, disease-free seedlings, and presumably with a high clonal fidelity (Nandhakumar et al. 2018; Khaskheli et al. 2021). In banana cultivation, this tool has been utilised for the *in vitro* regeneration of cultivars of commercial interest. For this purpose, various types of explants have been employed for both the induction of somatic embryos, *e.g.*, immature zygotic embryos (Escobedo-GraciaMedrano et al. 2014), proliferating meristem clumps called "scalps" (Schoofs et al. 1997), female flowers (Grapin et al. 2000), and immature male flowers (Côte et al. 1996; Enríquez-Valencia et al. 2019). The bottleneck of the somatic embryogenesis (SE) process in bananas has often been identified as the low germination rate and conversion into plantlets (Grapin et al. 1996). Therefore, various studies have examined the addition of plant growth regulators to promote efficient maturation of the somatic embryo (Khalil, Elbanna 2004; Kumaravel et al. 2017); however, this can result in low fidelity and clonal variation in the regenerated plants (Moradi et al. 2017). The considerable number of studies on somatic embryogenesis in bananas demonstrates that, similar to other plant species (Prado et al. 2010; Di Pauli et al. 2021), minor modifications to existing protocols, depending on the genotype or cultivar, significantly influence embryogenic callus formation, germination efficiency, and plant regeneration (Table S1 in Electronic Supplementray Material (ESM)). We previously reported a regeneration protocol through SE of the 'Manzano' cultivar (*Musa* spp. AAB, Silk subgroup), achieving a regeneration rate of somatic embryos close to 90% after 90 days of maturation, using a maturation medium free of plant growth regulators (Enríquez-Valencia et al. 2019). In this study, slight modifications were made to this *in vitro* regeneration protocol, which included a shorter period for the induction of cell suspensions and their filtration/screening using a 350 µm mesh, ultimately resulting in a high percentage of embryo germination and the production of plantlets of the 'Manzano' plantain and 'Pelipita' banana cultivars (*Musa* spp. AAB and *Musa* spp. ABB; cultivars from Silk and Bluggoe subgroups (Ruas et al. 2017), respectively).

MATERIAL AND METHODS

Induction and proliferation of embryogenic callus. For this study, immature male flowers of 'Manzano' plantain (*Musa* spp. AAB, Silk subgroup) and 'Pelipita' banana (*Musa* spp. ABB, Bluggoe subgroup) were collected in the experimental field of the Instituto Nacional de Investigaciones Forestales Agrícolas y Pecuarias (INIFAP), located in Uxmal, Yucatan, Mexico. The collected male buds were rinsed with water and then reduced to a length of 6–8 cm by successively removing the bracts. They were then immersed in 70% ethanol for 5 min and finally rinsed three times with sterile distilled water. Immature floral hands (sixth to 12th positions) from male flower buds were used to induce embryogenic callus, as previously described by Côte et al. (1996). Briefly, immature flowers were transferred to a glass jar with a capacity of 240 mL containing a semisolid medium (Ma 1991) prepared with Murashige and Skoog (MS) basal medium, including vitamins (Murashige, Skoog 1962), supplemented with biotin (4.1 µM), 2,4-D (18 µM), IAA (5.7 µM), NAA (5.4 µM), and sucrose (87.6 mM); the pH was adjusted to 5.7 with 1 N NaOH or HCl, before adding 2 g/L of gelrite and autoclaving at 120 °C for 20 minutes. Chemicals were purchased from PhytoTech Labs (PhytoTech Labs, Lenexa, KS, USA) or Sigma Aldrich (Sigma-Aldrich Co., St. Louis, MO, USA) unless otherwise noted. The cultures were incubated in the dark for 90 days at 25 ± 2 °C. After this period, the friable embryogenic callus was selected and transferred to flasks containing semi-solid proliferation medium (MP), composed of MS medium supplemented with 2,4-D (4.5 µM) and sucrose (87.6 mM). The pH was adjusted to 5.7 with 1 N NaOH (Faga Lab, Mocorito, Sin, Mex) or HCl (Merck, Darmstadt, Germany) before adding 2 g/L of gelrite and autoclaving at 120 °C for

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20 minutes. The cultures were incubated in darkness at 25 ± 2 °C. The explants were subcultured to fresh MP medium every month, and the necrotic tissue was removed to avoid oxidation of the embryogenic tissue.

Induction of cell suspensions and maturation of the somatic embryo. From embryogenic cultures, after two months, the formation of cell suspensions or suspension cultures was initiated by transferring 1.5 g of embryogenic callus to 50 mL of liquid medium (ML) composed of MS medium supplemented with biotin (4.1 µM), 2,4-D (4.5 µM), malt extract (100 mg/L), glutamine (680 mg/L), and sucrose (87.6 mM) into 250 mL Erlenmeyer flask (Côte et al. 1996). The pH was adjusted to 5.7 with 1 N NaOH or HCl before adding 2 g/L of gelrite and autoclaving at 120 °C for 20 minutes. The cultures were incubated under constant agitation at 90 rpm at 25 ± 2 °C in the dark. The cell suspensions were subcultured with 4% sedimented cell packs, renewing the medium every 7 days. After three rounds of subculturing, the suspension cultures were filtered through a 350 µm mesh, and the cells obtained from the filtration were washed with ML without plant growth regulators and finally transferred to Petri dishes containing semi-solid maturation medium (MM) composed of MS medium including MW vitamins (Morel, Wetmore 1951) and supplemented with KH_2PO_4 (1.25 mM) and sucrose (131.4 mM). The pH was adjusted to 5.7 with 1 N NaOH or HCl before adding 2 g/L of gelrite and autoclaving at 120 °C for 20 min (Enríquez-Valencia et al. 2019). It should be emphasised that the vitamins included in the MS medium were replaced by those reported by Morel and Wetmore (1951) only in the MM medium. The Petri dishes were incubated at 25 ± 2 °C in the dark. A stereomicroscope, Carl Zeiss SteREO Discovery.V8, coupled to a colour 105 Axiocam camera (Carl Zeiss Meditec AG, Jena, Germany), was used to observe the maturation process every 30 days.

Germination of somatic embryos. Approximately fifteen 60-day-old mature embryos at a late coleoptilar stage were selected and transferred to a glass jar containing the germination medium (GM), which was composed of MS medium and supplemented with KH_2PO_4 (1.25 mM) and sucrose (87.6 mM). The pH was adjusted to 5.7 with 1 N NaOH or HCl before adding 2 g/L of gelrite and autoclaving at 120 °C for 20 minutes. The cultures were initially incubated for 8 days in darkness and then transferred to a 16/8 h photoperiod with white light at 60 µE/m^2/s at 25 ± 2 °C. The germination percentage was determined when the first two leaves emerged, and the formation of roots was visually observed.

Statistical analysis. All experiments were conducted with three biological replicates, each consisting of 20 experimental units. Statistical analyses were performed by one-way ANOVA followed by Tukey's honest significant difference test ($P < 0.05$). GraphPad Prism (version 10, 2023) was used for all analyses.

RESULTS AND DISCUSSION

Research has shown that immature male flowers of bananas, specifically those positioned in places 4 to 18 from the apical meristem, can effectively induce the formation of embryogenic callus (Debbarma et al. 2019; Marimuthu et al. 2019). However, the efficiency and response time are influenced by the medium used to induce callus formation and can be highly genotype-specific; for certain cultivars, this period could extend up to eight months (Marimuthu et al. 2019). In light of this knowledge, harvested inflorescences of 'Manzano' and 'Pelipita' cultivars were dissected until the immature male flowers located in positions 6 to 12 from the apical meristem were exposed (Figure 1A, B, and 1E, F, respectively; see methods for details). Callus formation began a month after inoculating these immature male floral whorls in the induction medium (Figure 1C, G). After three months of culturing, embryogenic callus (EC) started to develop and continued to increase until the formation of pro-embryos. Approximately one to two months later, changes in EC morphology were observed, resulting in the appearance of compact callus (CC), translucent callus (TC), and necrotic callus (NC) (Figure 1D, H). Similar observations were reported for other banana cultivars, such as 'Prata-Anã' (Ribeiro et al. 2012; Souza Pádua et al. 2015) and 'Cavendish' ('Grand Naine' and 'Williams'; Youssef et al. 2010).

In our study, the induction frequency of EC was higher for the 'Manzano' cultivar than for the 'Pelipita' cultivar, with rates of 30% and 18%, respectively (Figure 2). Nonetheless, we demonstrated that a similar period of about 90 days is needed to induce callus formation in both cul-

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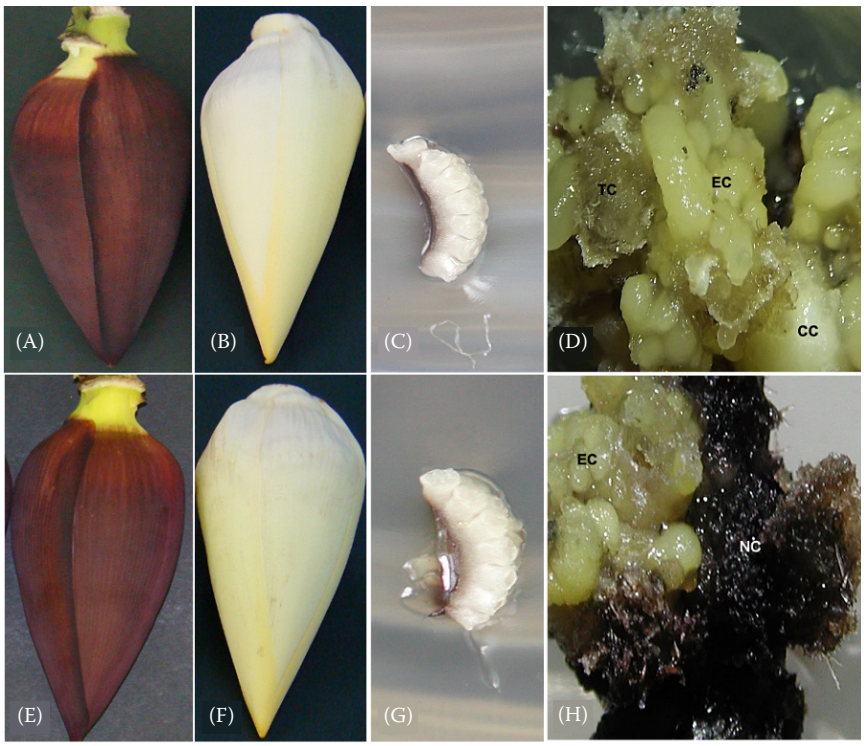

Figure 1. Callus induction using immature male-flower explants from 'Manzano' and 'Pelipita' banana cultivars (micrographs on top and bottom, respectively): (A) and (E) male flower buds; (B) and (F) partially reduced inflorescence; (C) and (G) immature male flowers used as explants and (D) and (H) different types of callus obtained after inducing its formation

EC – embryogenic callus; CC – compact callus; TC – translucent callus; NC – necrotic callus

tivars, following the methodology outlined here (see methods for details).

We used the pro-embryogenic mass formed on the surface of the white EC to establish cell suspension cultures, as reported by Enríquez-Valencia et al. (2019) and suggested by earlier studies (Côte et al. 1996; Ganapathi et al. 1999; Khalil, Elbanna 2004; Morais-Lino et al. 2016; Kumaravel et al. 2017; Ortiz Vargas et al. 2018). These EC were subcultured several times, then sieved and transferred to the maturation medium (Figure 3A, B, and G, H; see methods). Based on this prior knowledge, to obtain homogeneous Type II embryogenic aggregates characterised by a voluminous nucleus, a small vacuole, and the presence of small starch granules, 'Manzano' and 'Pelipita' cell suspensions were sifted through a 350 µm mesh (Figure 3C, I). This slight modification to our previous work, which used a mesh of 250 µm (Enríquez-Valencia et al. 2019), allowed for a better response in the maturation process and ultimately resulted in greater efficiency in embryo

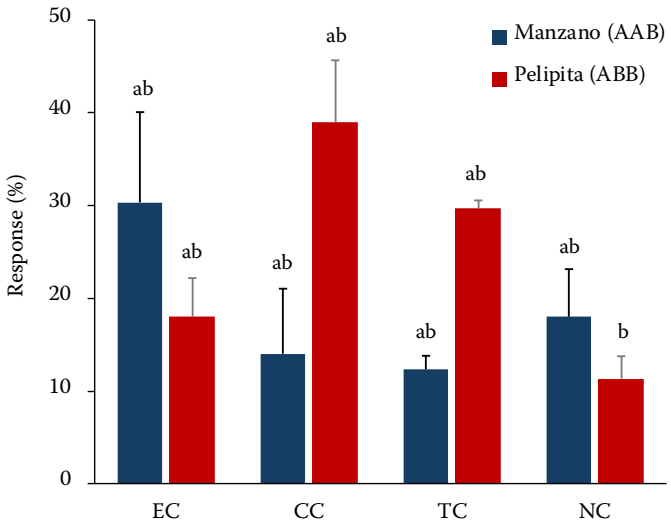

Figure 2. Response percentage in the formation of different types of callus for the 'Manzano' and 'Pelipita' cultivars

The *y*-axis shows response percentage, and the *x*-axis indicates callus type; EC – embryogenic callus; CC – compact callus; TC – translucent callus; NC – necrotic callus; data represent means ± SE of three biological replicates, each consisting of 20 experimental units; the different letters above the bars indicate statistically significant differences according to one-way ANOVA followed by Tukey's multiple-comparison test ($P \leq 0.05$)

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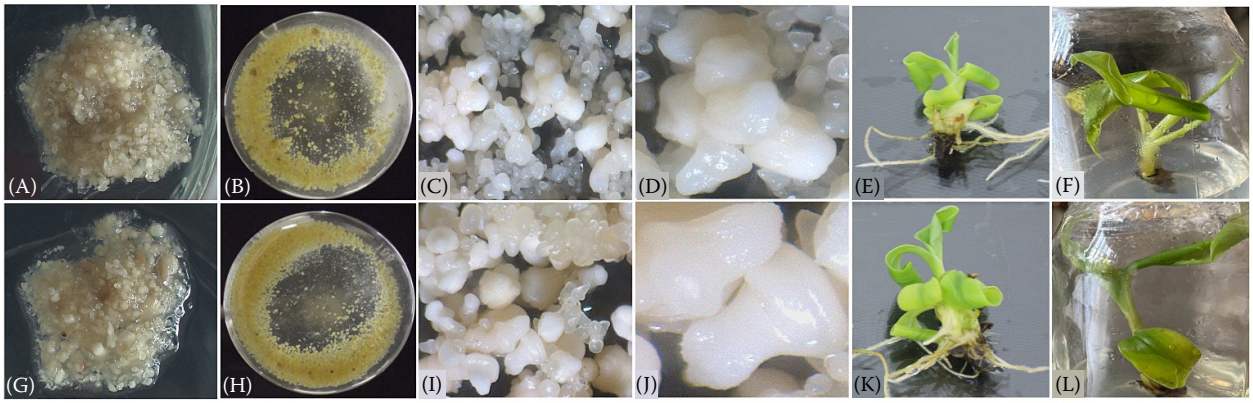

Figure 3. *In vitro* plant regeneration via indirect somatic embryogenesis of banana 'Manzano' and 'Pelipita' cultivars (micrographs on top and bottom, respectively): (A) and (G) the proliferation of embryogenic callus (30 dap); (B) and (H) cell suspension (15 dai); (C) and (I) 90-day-old mature somatic embryos; (D) and (J) embryos in late coleoptilar stage; (E) and (K) regenerated seedling (30 dag); (F) and (L) proliferated plants
Dap – days after proliferation; dai – days after inoculation; dag – days after germination

germination and *in vitro* plant regeneration (see Table 1). The sieving process is a critical step in the *in vitro* regeneration of *Musa* cultivars (Strosse et al. 2003). It has been reported that sieving cell suspensions and obtaining cells across a wide size range significantly affects the regeneration of somatic embryos. Côte *et al.* (1996) reported a low percentage of somatic embryo germination and *in vitro* plant regeneration (around 10%) and observed that after sieving cell suspensions for 80 days, the embryogenic aggregates in cell suspensions of the Grand Naine cultivar (*Musa* spp. AAA) were heterogeneous, with sizes ranging from 250 to 500 µm. Domergue et al. (2000), using the same cultivar ('Grand Naine'), identified five types of embryogenic aggregates in the cell suspensions (*ad verbatim*: "Type I corresponded to isolated cells or small cell aggregates. Type II was composed of embryogenic cells. Type III can be distinguished from Type II due to peripheral proliferation zones with embryonic cells. Type IV was composed of protodermic masses histologically comparable to proembryos. Type V were nodules composed of a central zone of meristematic cells and an external zone of starchy cells."). Type II aggregates, ranging from 50 to 800 µm, responded better in germination.

We observed the transition of somatic cells until their differentiation into somatic embryos. After 60 days, we noted distinct developmental stages of somatic embryogenesis in both the 'Manzano' and 'Pelipita' cultivars. These stages included translucent-white globular, scutellar, pear-shaped, and early and late coleoptilar stages (Figure 3D, J). Although our observations continued for 120 days, we found that 90 days of maturation was the optimal period to achieve the highest number of somatic embryos in the late coleoptilar stage. The duration of callus development until early shoot emergence was similar for both cultivars. Note that, although it is known that somatic embryos and zygotic embryos display similar patterns of developmental stages, such as globular, torpedo, and cotyledonary stages in dicots, or globular, scutellar, and coleoptilar stages in monocots, significant variation in somatic embryo formation has been observed among banana cultivars (Mordhorst et al. 1997; Debbarma et al. 2019; Enríquez-Valencia et al. 2019).

Interestingly, in the 'Manzano' and 'Pelipita' cultivars, after the globular stage of development, structures such as club-shaped embryo formations have been observed during the transition stage of embryo development, in addition to globular, pear-shaped, and early and late coleoptile embryos (Enríquez-Valencia et al. 2019). This is consistent with early observations that prove monocot crops produce spherical or club-shaped embryos without any tissue or organ differentiation (Johansen 1945). Moreover, the development of unique structures such as the scutellum, coleoptile, and several leaf primordia has also been reported in crops like barley (*Hordeum vulgare*; Norstog 1972; Engell 1989) and maize (*Zea mays*; van Lammeren 1986).

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Table 1. Germination percentage of somatic embryos (SE)

Genotype	SE germination at 60 days	SE germination at 90 days
	(%)	
Manzano (AAB)	88 ± 2[a]	93 ± 3[a]
Pelipita (ABB)	73 ± 3[b]	82 ± 2[b]

*Values represent mean ± standard error; superscript letters indicate significant differences ($P \leq 0.05$) according to Tukey test

However, there are no reports on the development and maturation process of the 'Pelipita' cultivar, marking the first report on this cultivar of interest.

Embryos in the late coleoptilar stage with an opaque white morphology were selected for transfer to the germination medium to evaluate the percentage of in vitro plant regeneration. Only those embryos that exhibited well-formed roots and leaves were considered germinated (Figure 3E, K). The germination percentage was assessed after 60 and 90 days. The 'Pelipita' cultivar showed a response percentage of 73 ± 3% at 60 days, and after 90 days, there was a slight increase to 82 ± 2% in the number of germinated embryos. In contrast, the 'Manzano' cultivar demonstrated a higher germination percentage, with 88 ± 2% at 60 days and 93 ± 3% at 90 days (Table 1). Although the differences in germination percentages between the two cultivars were relatively small, statistical analysis using a one-way ANOVA revealed that these differences were significant ($P < 0.05$). These results suggest that, while both cultivars responded favourably under the established culture conditions, physiological differences and genomic composition can influence the germination efficiency of somatic embryos. All germinated embryos converted to plantlets, producing complete and vigorous leaves (Figure 3F, L).

CONCLUSION

This study established an efficient protocol for somatic embryogenesis in 'Manzano' (AAB) and 'Pelipita' (ABB) using immature male flowers as initial explants. While the efficiency of embryogenic callus induction was higher in 'Manzano' than in 'Pelipita', both cultivars demonstrated a favourable response in the formation of embryogenic callus, which was then transferred to a liquid medium to promote the development of embryogenic cells. The induction of cell suspensions is a critical step in somatic embryogenesis, as it facilitates the formation of viable embryogenic cells, enabling rapid growth and uniform distribution in cell aggregates. However, the population of cell aggregates in suspension can adversely impact the later stages of embryonic development. Therefore, screening cell suspensions is crucial as it aims to homogenise the cell population, fostering a more uniform and efficient response during the maturation process of somatic embryos. By selecting type II cell aggregates, which possess desirable characteristics such as an optimal size range, the embryogenic potential of the culture is enhanced, reducing asynchronous development and benefiting the quality of somatic embryos during maturation. The reduction of growth regulators in the stages following callus induction, combined with the production of optimal suspension cells and the careful selection of maturation and germination media, resulted in high germination percentages of somatic embryos and regeneration efficiencies exceeding 80%. These results highlight the protocol's potential for large-scale micropropagation of cultivars threatened by pests and diseases, laying the groundwork for future applications in genetic improvement, conservation, and propagation programs for *Musa* species.

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REFERENCES

Adero M., Tripathi J.N., Tripathi L. (2023): Advances in somatic embryogenesis of banana. International Journal of Molecular Sciences, 24: 10999.

Aguilar Morán J. (2011): Improvement of Cavendish banana cultivars through conventional breeding. In: 7th Int. Symposium on Banana: ISHS-ProMusa Symposium

on Bananas and Plantains: Towards Sustainable Global Production. No. 986. ISHS: 205–208.

Assani A., Chabane D., Haïcour R., Bakry F., Wenzel G., Foroughi-Wehr B. (2005): Protoplast fusion in banana (*Musa* spp.): Comparison of chemical (PEG: polyethylene glycol) and electrical procedure. Plant Cell, Tissue and Organ Culture, 83: 145–151.

Becker D.K., Dugdale B., Smith M.K., Harding R.M., Dale J.L. (2000): Genetic transformation of Cavendish banana (*Musa* spp. AAA group) cv 'Grand naine' via microprojectile bombardment. Plant Cell Reports, 19: 229–234.

Côte F.X., Domergue R., Monmarson S., Schwendiman J., Teisson C., Escalant J.V. (1996): Embryogenic cell suspensions from the male flower of *Musa* AAA cv. Grand Naine. Physiologia Plantarum, 97: 285–290.

Crouch H., Crouch J., Madsen S., Vuylsteke D., Ortiz R. (2000): Comparative analysis of phenotypic and genotypic diversity among plantain landraces (*Musa* spp., AAB group). Theoretical and Applied Genetics, 101: 1056–1065.

Debbarma R., Sudhakar D., Kumar K.K., Soorianathasundaram K. (2019): Morphological and ultrastructure of developmental stages of somatic embryos of popular banana cultivars. International Journal of Current Microbiology and Applied Sciences, 8: 1676–1683.

Di Pauli V., Fontana P.D., Lewi D.M., Felipe A., Erazzú L.E. (2021): Optimized somatic embryogenesis and plant regeneration in elite Argentinian sugarcane (*Saccharum* spp.) cultivars. Journal of Genetic Engineering and Biotechnology, 19: 1–8.

Domergue F.G.R., Ferrière N., Côte F.X. (2000): Morphohistological study of the different constituents of a banana (*Musa* AAA, cv. Grand Naine) embryogenic cell suspension. Plant Cell Reports, 19: 748–754.

Drenth A., Kema G. (2021): The vulnerability of bananas to globally emerging disease threats. Phytopathology, 111: 2146–2161.

EFSA PLH Panel (EFSA Panel on Plant Health), Bragard C., Baptista P., Chatzivassiliou E., Di Serio F., Gonthier P., Jaques Miret J.A., Justesen A.F., MacLeod A., Magnusson C.S., Milonas P., Navas-Cortes J.A., Parnell S., Potting R., Stefani E., Thulke H.-H., Van der Werf W., Civera A.V., Yuen J., Zappalà L., Migheli Q., Vloutoglou I., Maiorano A., Streissl F., Reignault P.L. (2022): Pest categorisation of *Fusarium oxysporum* f.sp. *cubense* Tropical Race 4. EFSA Journal, 20: e07092.

Engell K. (1989): Embryology of barley: Time course and analysis of controlled fertilization and early embryo formation based on serial sections. Nordic Journal of Botany, 9: 265–280.

Enríquez-Valencia A.J., Vázquez-Flota F.A., Ku-Cauich J.R., Escobedo-GraciaMedrano R.M. (2019): Differentially expressed genes during the transition from early to late development phases in somatic embryo of banana (*Musa* spp. AAB group, Silk subgroup) cv. Manzano. Plant Cell, Tissue and Organ Culture, 136: 289–302.

Escobedo-GraciaMedrano R.M. Maldonado-Borges J.I., Burgos-Tan M.J., Valadez-González N., 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, 116: 175–185.

Escobedo-GraciaMedrano R.M., Enríquez-Valencia A.J., Youssef M., López-Gómez P., Cruz-Cárdenas C.I., Ku-Cauich J.R. (2016): Somatic embryogenesis in banana, *Musa* ssp.. In: Loyola-Vargas V., Ochoa-Alejo N. (eds): Somatic Embryogenesis: Fundamental Aspects and Applications. Cham, Springer: 381–400.

Etienne H. (2005): Somatic embryogenesis protocol: Coffee (*Coffea arabica* L. and *C. canephora* P.). In: Jain S.M., Gupta P.K. (eds): Protocol for Somatic Embryogenesis in Woody Plants. Forestry Sciences, Vol. 77, Dordrecht, Springer: 167–179.

FAOSTAT (2024): Food and Agriculture Data. Rome, FAO. Available at https://www.fao.org/faostat/en/#home (accessed Oct 24, 2024).

Florencio Anastasio J., Alarcon A., Garcia-Avila C., Ferrera-Cerrato R., Quezada Salinas A., Almaraz-Suárez J., Espinosa-Mendoza M., Bocanegra-Flores D., Ramos L. (2023): *In vitro* inhibition of bacteria against *Fusarium oxysporum* f. sp. *cubense* race 2. Mexican Journal of Phytopathology, 41: 126–142.

Ganapathi T.R., Suprasanna P., Bapat V.A., Kulkarni V.M., Rao P.S. (1999): Somatic embryogenesis and plant regeneration from male flower buds in banana. Current Science, 76: 1228–1231.

Ganapathi T.R., Negi S., Tak H., Bapat V.A. (2021): Transgenic banana: Current status, opportunities and challenges. In: Kavi Kishor P.B., Rajam M.V., Pullaiah T. (eds): Genetically Modified Crops. Vol 2, Singapore, Springer: 111–128.

García-Velasco R., Portal-González N., Santos-Bermúdez R., Yanes-Paz E., Lorenzo-Feijoo J.C., Companioni-González B. (2020): Fast method applied in previous evaluation of resistance of banana to *Fusarium oxysporum* f.sp. *cubense*. Mexican Journal of Phytopathology, 38: 384–397.

Grapin A., Schwendiman J., Teisson C. (1996): Somatic embryogenesis in plantain banana. Plant – In Vitro, 32: 66–71.

Grapin A., Ortíz J.-L., Lescot T., Ferrière N., Côte F.X. (2000): Recovery and regeneration of embryogenic cul-

https://doi.org/10.17221/35/2025-HORTSCI

tures from female flowers of False Horn Plantain. Plant Cell, Tissue and Organ Culture, 61: 237–244.

Häkkinen M. (2013): Reappraisal of sectional taxonomy in *Musa* (Musaceae). Taxon, 62: 809–813.

Inta W., Traiperm P., Ruchisansakun S., Janssens S.B., Viboonjun U., Swangpol S.C. (2023): Evolution and classification of Musaceae based on male floral morphology. Plants (Basel, Switzerland), 12: 1602.

Jalil M., Khalid N., Yasmin Othman R. (2003): Plant regeneration from embryogenic suspension cultures of *Musa acuminata* cv. Mas (AA). Plant Cell, Tissue and Organ Culture, 75: 209–214.

Johansen D.A. (1945): A critical survey of the present status of plant embryology. The Botanical Review, 11: 87–107.

Justine A.K., Kaur N., Savita, Pati P.K. (2022): Biotechnological interventions in banana: Current knowledge and future prospects. Heliyon, 8: e11636.

Kamada H., Kobayashi K., Kiyosue T., Harada H. (1989): Stress induced somatic embryogenesis in carrot and its application to synthetic seed production. *In vitro* Cellular & Developmental Biology, 25: 1163–1166.

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

Khaskheli A.J., Ali M., Shah S.Z.H., Memon Z.F., Awan S., Khaskheli M.I., Khaskheli M.A., Magsi B., Qambrani Z., Khaskheli A.A. (2021): Initiation, proliferation, and improvement of a micropropagation system for mass clonal production of banana through shoot-tip culture. Journal of Plant Biotechnology, 48: 86–92.

Kumaravel M., Uma S., Backiyarani S., Saraswathi M.S., Vaganan M.M., Muthusamy M., Sajith K.P. (2017): Differential proteome analysis during early somatic embryogenesis in *Musa* spp. AAA cv. Grand naine. Plant Cell Reports, 36: 163–178.

Kumari P., Gaur S.S., Tiwari R.K. (2023): Banana and its by-products: A comprehensive review on its nutritional composition and pharmacological benefits. eFood, 4: e110.

Lu C., Vasil I., Ozias-Akins P. (1982): Somatic embryogenesis in *Zea mays* L. Theoretical and Applied Genetics, 62: 109–112.

Ma S. (1991): Somatic embryogenesis and plant regeneration from cell suspension culture of banana. In: Proc. Symposium on Tissue Culture of Horticultural Crops. Taipei, March 8–9, 1988: 181–188.

Marimuthu K., Subbaraya U., Suthanthiram B., Marimuthu S.S. (2019): Molecular analysis of somatic embryogenesis through proteomic approach and optimization of protocol in recalcitrant *Musa* spp. Physiologia Plantarum, 167: 282–301.

Méndez-Hernández H.A., Ledezma-Rodríguez M., Avilez-Montalvo R.N., Juárez-Gómez Y.L., Skeete A., Avilez-Montalvo J., De-la-Peña C., Loyola-Vargas V.M. (2019): Signaling overview of plant somatic embryogenesis. Frontiers in Plant Science, 10: 1–15.

Molina D.M., Aponte M.E., Cortina H., Moreno G. (2002): The effect of genotype and explant age on somatic embryogenesis of coffee. Plant Cell, Tissue and Organ Culture, 71: 117–123.

Moradi Z., Farahani F., Sheidai M., Nejad Satari T. (2017): Somaclonal variation in banana (*Musa acuminate* Colla cv. Valery) regenerated plantlets from somatic embryogenesis: Histological and cytogenetic approaches. Caryologia, 70: 1–6.

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

Mordhorst A.P., Toonen M.A.J., de Vries S.C., Meinke D. (1997): Plant embryogenesis. Critical Reviews in Plant Sciences, 16: 535–576.

Morel G., Wetmore R.H. (1951): Tissue culture of monocotyledons. American Journal of Botany, 38: 138–140.

Murashige T., Skoog F. (1962): A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia Plantarum, 15: 473–497.

Nandhakumar N., Kumar K., Sudhakar D., Soorianathasundaram K. (2018): Plant regeneration, developmental pattern and genetic fidelity of somatic embryogenesis derived *Musa* spp. Journal of Genetic Engineering and Biotechnology, 16: 587–598.

Norstog K. (1972): Early development of the barley embryo: Fine structure. American Journal of Botany, 59: 123–132.

Olivares B.O., Rey J.C., Lobo D., Navas-Cortés J.A., Gómez J.A., Landa B.B. (2021): *Fusarium* wilt of bananas: A review of agro-environmental factors in the Venezuelan production system affecting its development. Agronomy, 11: 986.

Ortiz Vargas J.L., Sandoval Fernández J., Aguilar Vega M.E. (2018): Regeneration of plants from embryogenic cell suspensions of cv. 'Datil' (*Musa* AA): Morphological evaluation of plants in the field. Journal of Agricultural Science and Technology B, 8: 29–41.

Ploetz R.C. (2015): *Fusarium* wilt of banana. Phytopathology, 105: 1512–1521.

Prado M.J., Grueiro M.P., González M.V., Testillano P.S., Domínguez C., López M., Rey M. (2010): Efficient plant regeneration through somatic embryogenesis from anthers and ovaries of six autochthonous grapevine cultivars from Galicia (Spain). Scientia Horticulturae, 125: 342–352.

https://doi.org/10.17221/35/2025-HORTSCI

Qazi J. (2016): Banana bunchy top virus and the bunchy top disease. Journal of General Plant Pathology, 82: 2–11.

Ribeiro L.O., Vilela Paiva L., Souza Pádua M., Régis Santos B., Alves E., Stein V.C. (2012): Morphological and ultrastructural analysis of various types of banana callus, cv. Prata Anã. Acta Scientiarum. Agronomy, 34: 423–429.

Rowe P., Rosales F. (1994): Musa Breeding at FHIA. The Improvement and Testing of Musa: A Global Partnership. INIBAP, Montpellier:117–129.

Ruas M., Guignon V., Sempere G., Sardos J., Hueber Y., Duvergey H., Andrieu A., Chase R., Jenny C., Hazekamp T., Irish B., Jelali K., Adeka J., Ayala-Silva T., Chao C.P., Daniells J., Dowiya B., Effa Effa B., Gueco .L, Herradura L., Ibobondji L., Kempenaers E., Kilangi J., Muhangi S., Ngo Xuan P., Paofa J., Pavis C., Thiemele D., Tossou C., Sandoval J., Sutanto A., Vangu Paka G., Yi G., Van den Houwe I., Roux N., Rouard M. (2017): MGIS: Managing banana (*Musa* spp.) genetic resources information and high-throughput genotyping data. Database. The Journal of Biological Databases and Curation, 2017: bax046.

Sági L, Panis B., Remy S., Schoofs H., Smet K.D., Swennen R., Cammue B.P. (1995): Genetic transformation of banana and plantain *(Musa* spp.) via particle bombardment. Biotechnology, 13: 481–485.

Schoofs H., Panis B., Swennen R. (1997): Competence of scalps for somatic embryogenesis in *Musa*. In: 2nd Int. Symp. Banana: 1st Int. Symp. Banana in the Subtropics. ISHS Acta Horticulturae, 490: 475–484.

Souza Pádua M., Lima C.D., Paiva V.L., Barduche D., Régis Santos B., Stein C.V. (2015): Histological and ultrastructural analysis of the banana cv. Prata-Anã embryogenic calluses and cell suspension. Revista de Ciências Agrárias – Amazonian Journal of Agricultural and Environmental Sciences, 58: 168–175.

Strosse H., Domergue R., Panis B., Vézina A., Escalant J.V., Picq C., Côte F. (2003): Banana and plantain embryogenic cell suspensions. In: Vézina A., Picq C. (eds): INIBAP Technical Guidelines 8. Montpellier, The International Network for the Improvement of Banana and Plantain: 1–31.

Subramanyam K., Subramanyam K., Sailaja K.V., Srinivasulu M., Lakshmidevi K. (2011): Highly efficient *Agrobacterium*-mediated transformation of banana cv. Rasthali (AAB) via sonication and vacuum infiltration. Plant Cell Reports, 30: 425–436.

Tinzaara W., Mutambuka M., Oyesigye E., Blomme G., Dita M., Gold C.S., Rouard M., Karamura E. (2024): Banana wilt diseases: Current status and future research strategies for their management. International Journal of Pest Management, 70: 290–309.

Tripathi L., Ntui V.O., Tripathi J.N. (2024): Application of CRISPR/Cas-based gene-editing for developing better banana. Frontiers in Bioengineering and Biotechnology, 12: 1395772.

van Lammeren A.A.M. (1986): Developmental morphology and cytology of the young maize embryo (*Zea mays* L.). Acta Botanica Neerlandica, 35: 169–188.

Wang Z., Miao H., Liu J., Xu B., Yao X., Xu C., Zhao S., Fang X., Jia C., Wang J., Zhang J., Li J., Xu Y., Wang J., Ma W., Wu Z., Yu L., Yang Y., Liu C., Guo Y., Sun S., Baurens F.C., Martin G., Salmon F., Garsmeur O., Yahiaoui N., Hervouet C., Rouard M., Laboureau N., Habas R., Ricci S., Peng M., Guo A., Xie J., Li Y., Ding Z., Yan Y., Tie W., D'Hont A., Hu W., Jin Z. (2019): *Musa balbisiana* genome reveals subgenome evolution and functional divergence. Nature Plants, 5: 810–821.

Yadav A. (2021): Banana (*Musa acuminata*): Most popular and common Indian plant with multiple pharmacological potentials. World Journal of Biology Pharmacy and Health Sciences, 7: 036–044.

Yonow T., Ramirez-Villegas J., Abadie C., Darnell R.E., Ota N., Kriticos D.J. (2019): Black Sigatoka in bananas: Ecoclimatic suitability and disease pressure assessments. PLoS One, 14: e0220601.

Youssef M., James A., Mayo-Mosqueda A., Ku-Cauich J.R., Grijalva-Arango R., Escobedo-G.M.R.M. (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: 2216–2223.

Zimmerman J.L. (1993): Somatic embryogenesis: A model for early development in higher plants. The Plant Cell, 5: 1411–1423.

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