



Jul 30, 2025

Development of a Thin-Layer Liquid Culture-Based Corm Induction System for Cavendish Banana (Musa spp. AAA group)

DOI

<https://dx.doi.org/10.17504/protocols.io.bp2l6zb5dgqe/v1>

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Protocol Citation: Pradeep C Deo 2025. Development of a Thin-Layer Liquid Culture-Based Corm Induction System for Cavendish Banana (Musa spp. AAA group). **protocols.io** <https://dx.doi.org/10.17504/protocols.io.bp2l6zb5dgqe/v1>

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Protocol status: Working

We use this protocol and it's working

Created: July 28, 2025

Last Modified: July 30, 2025

Protocol Integer ID: 223400

Keywords: corm formation in cavendish banana, corm induction system for cavendish banana, propagating banana, cavendish banana, cultured in liquid ms medium, layer liquid culture, musa spp, vitro shoot, layerliquid culture system, rhizome crops like taro, simpler germplasm movement, rhizome crop, formed corm, corm formation, turmeric, suitable for direct soil transfer, direct soil transfer, meristematic tissue, based corm induction system

Funders Acknowledgements:

Bill & Melinda Gates Foundation

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Abstract

This study describes a thin-layerliquid culture system for in vitro corm formation in Cavendish banana (*Musa* spp. AAA group). Explants were cultured in liquid MS medium with varying concentrations of sucrose (40 or 60 g/L) and BAP (0, 2, or 5 mg/L). Corms formed only in the presence of BAP, with the largest observed in 60 g/L sucrose and 5 mg/L BAP. These in vitro-formed corms contained meristematic tissue and were capable of both shoot and root development upon transfer to hormone-free medium. Unlike fragile in vitro shoots, corms are robust, easier to handle, and suitable for direct soil transfer with minimal acclimatisation. This method offers a practical alternative for propagating banana and other corm/rhizome crops like taro, ginger, and turmeric, and facilitates safer, simpler germplasm movement.

Overview

- 1 This protocol describes a thin-layer liquid culture system optimized for rapid and efficient corm induction in Cavendish banana. It outlines key steps, media compositions, and culture conditions suitable for improving in vitro propagation outcomes.

Abstract

- 2 This study describes a thin-layer liquid culture system for in vitro corm formation in Cavendish banana (*Musa* spp. AAA group). Explants were cultured in liquid MS medium with varying concentrations of sucrose (40 or 60 g/L) and BAP (0, 2, or 5 mg/L). Corms formed only in the presence of BAP, with the largest observed in 60 g/L sucrose and 5 mg/L BAP. These in vitro-formed corms contained meristematic tissue and were capable of both shoot and root development upon transfer to hormone-free medium. Unlike fragile in vitro shoots, corms are robust, easier to handle, and suitable for direct soil transfer with minimal acclimatisation. This method offers a practical alternative for propagating banana and other corm/rhizome crops like taro, ginger, and turmeric, and facilitates safer, simpler germplasm movement.

Introduction

- 3 Vegetatively propagated crops such as banana (*Musa* spp.), taro (*Colocasia esculenta*), ginger (*Zingiber officinale*), and turmeric (*Curcuma longa*) rely on underground storage organs like corms and rhizomes for multiplication. While conventional plant tissue culture methods have significantly improved propagation rates, in vitro-grown plantlets often remain fragile and poorly suited to withstand the rigours of acclimatisation, transport, and field transfer (Yamamoto & Matsumoto, 1992; Medina et al., 2009).

Inducing corms or rhizomes in vitro offers several advantages over traditional shoot-based propagation:

- Reduces dependency on lengthy acclimatisation. In vitro-formed corms are physiologically more robust and better equipped to survive in non-sterile environments (Zhou et al., 1999).
- Enhances survivability during handling and transport. Unlike delicate shoots, corms are compact and resilient, reducing physical damage and contamination risks (Hu et al., 2006).
- Simplifies germplasm exchange since small, dense propagules like corms and rhizomes can be shipped without specialized packaging or gel-based media (Medina et al., 2009).

- Speeds up field deployment since their structural and metabolic maturity supports direct pot-to-field transfer, ideal for large-scale propagation programs (Yamamoto & Matsumoto, 1992).

Previous studies have demonstrated the successful induction of corms in taro and saffron using high sucrose concentrations (6–10%) and elevated levels of BAP (5–22 μ M) in liquid or semi-solid culture systems (Yamamoto & Matsumoto, 1992; Zhou et al., 1999; Medina et al., 2009; Hu et al., 2006). However, protocols tailored specifically for in vitro corm formation in Cavendish banana remain underexplored.

This study aimed to develop a thin-layer liquid culture system for in vitro corm formation in Cavendish banana, with the goal of generating compact, robust propagules containing meristematic tissue. These in vitro-formed corms can be transferred directly to pots with soil, minimizing acclimatisation time and facilitating efficient field establishment.

Materials

- 4
 - In vitro Cavendish banana plantlets (5 cm tall)
 - 250 mL Erlenmeyer flasks
 - 50 mL Falcon tubes
 - 0.22 μ m syringe filters
 - 20 mL sterile syringes
 - 1.5 mL sterile microfuge tubes
 - Schott bottles (for media)
 - Micro-pipettes (P1000, P200, P20)
 - Sterile pipette tips (for each pipette)
 - Forceps and scalpel (sterile)
 - Laminar airflow cabinet
 - Aluminum foil (for flask sealing)

Reagents

- 5

Note: All reagents were purchased from Sigma-Aldrich (now Merck) unless otherwise specified.

 - MS Basal Salt Mixture (M5524; Sigma-Aldrich)
 - BAP (6-Benzylaminopurine; Sigma-Aldrich)
 - 1 M NaOH & 1 M HCl (for pH adjustment)
 - Glycine
 - Nicotinic acid
 - Pyridoxine-HCl
 - Thiamine-HCl



- Sucrose
- Distilled water (Milli-Q or equivalent)

Media & Reagents Preparation

6 1. BAP Stock Solution (1 mg/mL, 20 mL)

1. Weigh 0.02 g of BAP powder on a clean weigh tray.
2. Transfer the BAP powder into a sterile 50 mL Falcon tube.
3. Add a few drops of 1 M NaOH to aid dissolution. Mix gently by swirling or vortexing.
4. Once fully dissolved, make up the final volume to 20 mL with sterile distilled water.
5. Filter sterilize the solution using a 0.22 μ m syringe filter.
6. Aliquot 1000 μ L (1 mL) into sterile 1.5 mL microfuge tubes.
7. Store the aliquots at -20°C until use.

2. Vitamin Stock Solution (500X, 500 mL)

1. Weigh the following components into a weigh tray individually:
 - Glycine: 0.5 g
 - Nicotinic acid: 0.125 g
 - Pyridoxine-HCl: 0.125 g
 - Thiamine-HCl: 0.1 g
2. Transfer the powders into a clean 500 mL beaker containing 350–400 mL of distilled water.
3. Stir with a magnetic stirrer until fully dissolved.
4. Carefully transfer the solution into a 500 mL volumetric flask.
5. Rinse the beaker with a small amount of distilled water to collect any remaining vitamins and add the rinse to the volumetric flask.
6. Bring the final volume to 500 mL with distilled water.
7. Store at 4°C , preferably protected from light.

To Use: Add 2 mL of this 500X stock per 1 L of medium before autoclaving.

Tip: A 500X stock minimizes the volume added per litre and simplifies weighing with convenient gram amounts.

3. MS Medium with 30g/L sucrose

1. In a clean 1 L beaker, add 700–800 mL of distilled water.
2. Weigh and add 4.33 g of MS basal salt mixture (M5524).
3. Stir with a magnetic stirrer until the salts dissolve completely.
4. Add 30 g of sucrose and continue stirring until fully dissolved.
5. Add 2 mL of 500X vitamin stock solution.
6. Adjust the pH to 5.7 using 1 M NaOH.
7. Bring the final volume up to 1 L with distilled water.
8. Dispense into appropriate vessels and autoclave at 121°C for 15 minutes.

9. Once cooled, store the medium in the dark at room/root temperature (23–24 °C).

Storage Tip:

Autoclaved medium can be stored for 4–6 weeks in a cool, dark environment to maintain quality.

4. Corm Initiation Medium (CIM)

Note: Prepare two sets of 1.5 L MS medium with different sucrose concentrations: 40 g/L and 60 g/L.

1. In two separate 2 L beakers, add 1 L of distilled water to each.
2. Weigh and add 6.5 g of MS basal salt mixture (M5524, Sigma-Aldrich) to each beaker.
3. Add:
 - 40 g/L Sucrose media → Add 60 g sucrose and label this beaker “40 g/L Sucrose”.
 - 60 g/L Sucrose media → Add 90 g sucrose and label this beaker “60 g/L Sucrose”.
4. Add 2 mL of 500X vitamin stock to each beaker.
5. Stir until all components are completely dissolved.
6. Adjust pH to 5.7 using NaOH or HCl.
7. Make up the final volume of each to 1.5 L with distilled water.
8. Label six clean 500 mL Schott bottles as T1 to T6.
9. Dispense:
 - T1, T2, T3: 500 mL each from the 40 g/L sucrose medium.
 - T4, T5, T6: 500 mL each from the 60 g/L sucrose medium.
10. Autoclave all bottles at 121 °C for 15 minutes.
11. Allow media to cool to 40–50 °C before adding BAP. Use 1 mg/mL BAP stock (filter-sterilized) and add as outlined in Table 1 below :
12. Store the media in the dark at 23–24 °C for 3–4 weeks.

Table 1: Treatment Combinations for In Vitro Corm Formation in Cavendish Banana Using Varying Sucrose and BAP Concentrations

Bottle	Sucrose	BAP Addition	Final BAP Concentration
T1	40 g/L	None	0 mg/L (Control)
T2	40 g/L	1 mL	2 mg/L
T3	40 g/L	2.5 mL	5 mg/L
T4	60 g/L	None	0 mg/L (Control)
T5	60 g/L	1 mL	2 mg/L
T6	60 g/L	2.5 mL	5 mg/L

Each treatment (T1–T6) represents a unique combination of sucrose concentration (40 or 60 g/L) and 6-benzylaminopurine (BAP) addition to assess its effect on corm formation. The final BAP concentration in the medium was adjusted by adding either 1 mL (2 mg/L) or 2.5 mL (5 mg/L) of BAP stock solution, while controls received no BAP.

Step-by-step procedure

- 7 Refer to the schematic flow diagram in Figure 1 for a visual overview of the protocol timeline and key treatment phases.

Caution: All processes should be carried out in a Laminar Air Flow (LAF) Hood.

- 8 MS-Murashige & Skoog (1962); BAP-6-benzylaminopurineMS-Murashige & Skoog (1962); BAP-6-benzylaminopurine
Step 1: Explant Preparation and Inoculation (Day 0)
 1. Select healthy in vitro Cavendish banana plantlets approximately 5 cm tall.
 2. Using a sterile scalpel, remove the leaves along with one-third of the petiole and trim off two-thirds of the corm base, leaving an explant of approximately 2–3 cm in length.
 3. Transfer 25 mL of Corm Induction Medium (CIM) into sterile 250 mL Erlenmeyer flasks for each of the six treatment combinations (T1–T6), as described in Table 1. Each treatment represents a unique combination of sucrose (40 or 60 g/L) and BAP (0, 2, or 5 mg/L).

Note: Prepare three replicate flasks for each treatment, resulting in a total of 18 flasks (6 treatments × 3 replicates).

4. Under sterile conditions in a laminar flow hood, place five prepared explants into each flask, totalling 15 explants per treatment .

5. Seal flasks with sterile aluminium foil caps.
6. Place the flasks under the following conditions:
 - Photoperiod: 16 hours light / 8 hours dark
 - Temperature: 24–26 °C
 - Flasks: Stationary (not shaken)

Step 2: Corm Induction Phase

(Weeks 0–6)

7. Incubate flasks for 3 weeks without subculturing.
8. At Week 3, remove spent media under sterile conditions and add 25 mL of fresh CIM (same sucrose and BAP treatment).
9. Continue incubation under the same conditions for another 3 weeks.

Step 3: Recovery and Root Induction (Weeks 6–8)

10. At Week 6, carefully remove CIM and replace it with 25 mL of hormone-free MS medium in all flasks.
11. Continue culture for 2 additional weeks to allow:
 - Leaching of excess BAP
 - Restoration of osmotic balance
 - Root and shoot development.

Note: Follow the timeline and treatment transitions shown in Figure 1 to ensure optimal results and reproducibility.

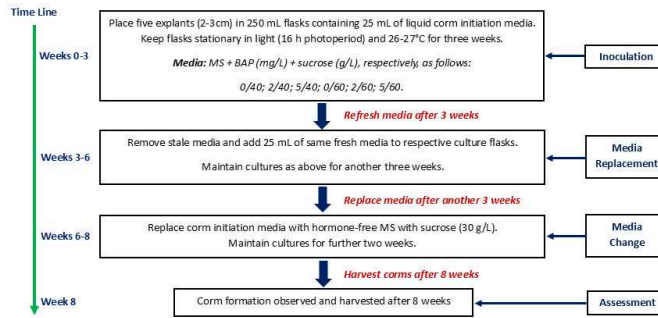


Figure 1: The following schematic outlines the step-by-step protocol used for the induction and development of corms in banana explants under in vitro conditions. Researchers are advised to closely follow the timeline and media treatments detailed in the figure below to ensure reproducibility and optimal corm formation. Each step, from explant inoculation to final assessment, is clearly indicated along a structured 8-week timeline.

MS-Murashige & Skoog (1962); BAP-6-benzylaminopurine

Expected Results and Discussions

- 9 Banana plantlets cultured in liquid MS medium supplemented with varying concentrations of sucrose and BAP displayed distinct morphological responses, particularly at the basal region (Figure 2). In the absence of BAP, explants developed elongated roots with dense root hairs, but no basal swelling was observed, regardless of sucrose concentration.

These results suggest that sucrose alone is insufficient to trigger corm-like structure formation in banana. Instead, it supports general root development, which is consistent with previous observations in taro and ginger, where auxin-dominant or hormone-free media favoured rooting over organogenic or storage tissue formation (Yamamoto & Matsumoto, 1992; Swarnathilaka et al., 2018).

In contrast, explants cultured on media containing both high sucrose and BAP showed prominent basal swelling, a key indicator of corm induction. Root development was completely suppressed during the treatment period. The explants exhibited stunted shoot growth and occasional leaf senescence, indicating that high cytokinin levels, while promoting corm initiation, temporarily suppress apical dominance and

vegetative growth. Similar patterns have been observed in taro, orchids, and saffron, where elevated BAP combined with 6–10% sucrose promoted micro-corm formation but also led to reduced shoot elongation and early leaf senescence (Zhou et al., 1999; Medina et al., 2009; Sharma et al., 2007).

Upon transfer to hormone-free MS medium, all BAP-treated explants resumed root development, indicating that the cytokinin-induced inhibition is reversible and that rooting can be effectively separated from corm induction.



Figure 2: *In vitro* corm formation in Cavendish banana cv. Williams. Explants were cultured in MS liquid medium supplemented with different concentrations of sucrose and 6-benzylaminopurine (BAP) for six weeks, followed by two weeks in hormone-free MS. Treatments included sucrose at 60 g/L with BAP at: (A) 0 mg/L, (B) 2 mg/L, (C) 5 mg/L; and sucrose at 40 g/L with BAP at: (D) 0 mg/L, (E) 2 mg/L, (F) 5 mg/L. Each treatment had three biological replicates with five explants per replicate. Corm formation was enhanced in the presence of BAP, with the largest and most uniform corms observed in the 60 g/L sucrose + 5 mg/L BAP treatment. Explants without BAP developed roots but showed no basal swelling. The figure illustrates differences in corm size and development under each treatment.

Culturing in hormone-free medium facilitates the leaching and dilution of residual BAP, thereby reducing its inhibitory effects on rooting and overall plant development. This two-phase culture system aligns with strategies used in *in vitro* micro-rhizome production in ginger, where rhizome induction occurs under high BAP and sucrose concentrations, followed by rooting in hormone-free or auxin-supplemented conditions (Yu et al., 2024; David et al., 2019).

The most significant corm development was observed in explants treated with 60 g/L sucrose and 5 mg/L BAP, which produced large, compact, and uniform corms. This

outcome indicates a synergistic effect between high osmotic potential and cytokinin concentration, as also reported in in vitro systems of taro and saffron (Hu et al., 2006; Zhou et al., 1999). BAP likely plays a dominant role in promoting cell division and meristem organization at the base, while high sucrose supports energy-intensive storage tissue development and thickening (Swarnathilaka et al., 2018; Yamamoto & Matsumoto, 1992).

Overall, the combination of 5 mg/L BAP and 60 g/L sucrose is expected to be the most effective for inducing compact, meristem-rich corms suitable for direct potting and field establishment. These findings highlight the potential of targeted PGR-sucrose regimes to optimize in vitro corm production in banana and other vegetatively propagated crops.

Conclusion

- 10 A thin-layer liquid culture system enriched with 60 g/L sucrose and 5 mg/L BAP effectively induces in vitro corms in Cavendish banana. This technique shows promise for rapid propagation and field establishment in banana and other corm/rhizome-based crops like taro, ginger, and turmeric, while also streamlining germplasm transport and handling.

Troubleshooting

- 11

Problem 1: No corm formation

Possible Cause: Absence or insufficient concentration of BAP

Solution: Ensure BAP is added at the correct concentration (2–5 mg/L). Use freshly prepared stock solutions and filter sterilize before adding to the medium.

Problem 2: Root formation instead of corm development

Possible Cause: Hormone-free or auxin-dominant media conditions

Solution: Use cytokinin-enriched medium (with BAP) during the corm induction phase. Avoid auxins or hormone-free media at this stage.

Problem 3: Contamination in culture flasks

Possible Cause: Poor aseptic technique or inadequate sterilization

Solution: Work strictly under a laminar flow hood. Sterilize all tools, flasks, and media thoroughly before use.

Problem 4: Explant browning or necrosis

Possible Cause: Osmotic stress from high sucrose or cytokinin toxicity

Solution: Monitor media composition. If necrosis occurs, reduce sucrose concentration slightly or shorten exposure time before transfer to hormone-free

medium.

Problem 5: Poor or no rooting in recovery phase

Possible Cause: Residual BAP in tissues after induction phase

Solution: Ensure transfer to hormone-free MS medium is done at the correct time (Week 6). Allow at least 2 weeks for BAP leaching and osmotic balance restoration.

Note: Not all explants will form roots immediately after transfer. Some may require slightly longer to leach out residual BAP before root initiation occurs—this is normal.

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