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RESEARCH ARTICLE

# Morpho-anatomy of diploid and triploid *Musa* cultivars CO 2 and CO 3 male inflorescence and its implications in micropropagation

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## Abstract

Banana (*Musa* spp.) is a major staple fruit and cash crop globally cultivated, particularly in tropical and subtropical countries. It exhibits a complex inflorescence, which plays a vital role in its reproductive process. The inflorescence of bananas by distinct morphological and anatomical features that vary among different species and cultivars. Tissue culture techniques have emerged as pivotal tools that offer rapid propagation methods, utilising various explants to meet growing demands and enhance crop resilience. This study evaluated the morphological and anatomical characteristics of *Musa paradisiaca* cultivars CO 2 and CO 3, focusing on their potential as explants in tissue culture. Key differences between these two lines include floral axis orientation, bract pigmentation, flower structure and flower quantity. Notably, CO 2 exhibited the presence of papillae, whereas CO 3 lacked them. Both cultivars contained calcium oxalate crystals and raphides, which define their distinct anatomical traits and enhance their suitability for micropropagation. Furthermore, tissue culture experiments demonstrated early greening, faster callus formation and efficient shoot regeneration, with CO 2 demonstrating a slightly superior response to CO 3. The male inflorescences of both cultivars, when cultivated in Murashige and Skoog (MS) media, responded within a greening time of approximately 16.2 days, swelling within 28.4 days and bud formation ranging from 2 to 6 per cluster, leading to the production of 12 to15 per nodal cluster. These findings suggest that male inflorescences have significant potential for efficient micropropagation, providing a valuable resource for banana cultivation and genetic improvement.

## Keywords

calcium oxalate; male inflorescence; morpho-anatomy; *Musa paradisiaca*; raphides; tissue culture

## Introduction

Banana (*Musa* spp.) is a major staple fruit crop worldwide, especially in tropical and subtropical regions. Its holds significant economic and cultural importance, serving as both dietary staple and a cash crop for millions of people (1, 2). Most edible bananas are derived from natural hybrids of *Musa acuminata* (AA) and *Musa balbisiana* (BB), resulting in a wide array of cultivars adapted to diverse

agroclimatic conditions (3, 4). The genetic diversity and adaptability of theses cultivars have been well documented, offering key insights into their morphological and reproductive traits (5).

In India, indigenous varieties such as Ney Poovan and Karpooravalli are highly valued for their flavor and adaptability. However, they face increasing challenges from pests, diseases and abiotic stressors such as drought and wind. Over the years, the classification of banana cultivars has been refined through morphological and genomic insights (6).

The cultivars CO 2 and CO 3, developed by Tamil Nadu Agricultural University (TNAU), are promising hybrids with distinct characteristics and enhanced resistance to pests and diseases. CO 2, derived from the cross *Karpooravalli* (ABB) × *Pisang Lilin* (AA) and CO 3, a hybrid of *Karpooravalli* (ABB) × *H201* (AB), have been widely adopted in Southern India. However, their morpho-anatomical traits, particularly those of male inflorescences, have not yet been studied.

Although plant tissue culture has been explored extensively in bananas, the use of male inflorescences as explants remains underexplored, primarily due to limited information of their anatomical features. Tissue culture techniques have emerged as pivotal tools that offer rapid propagation methods to meet growing demands and enhance crop resilience.

Among various conventional explants, such as sword suckers, rhizome tissues, in male inflorescence offers a distinct advantage due to their non-destructive nature, allowing propagation without compromising the integrity of the mother plant (7). The banana inflorescence, or "banana heart," exhibits unique morphological and anatomical features that vary across cultivars and hold potential for efficient micropropagation. Features, including the vascular system, bract structure and flower arrangement, influence its suitability as an explant in tissue cultures.

This study aimed to analyze the morphological and anatomical characteristics of male inflorescences from the CO 2 and CO 3 cultivars to assess their potential as explants in tissue culture. By understanding these traits, this study contributes to characterization better understanding of banana cultivars and enhances the prospects for efficient and sustainable micropropagation techniques.

## Materials and Methods

### *Plant materials*

The aerial male inflorescences of *Musa paradisiaca* L., (Musaceae) (cv.) CO 2 and CO 3 were collected four months before harvesting from Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, India (11.0096° N, 76.9294° E). The male inflorescences were harvested from the distal end of the pseudostem after the corresponding fruit bunch had fully developed.

These inflorescences were selected as explants for tissue culture due to their degenerative nature and ease of handling compared to sword suckers. Three inflorescences from each cultivar were sampled, with sections obtained from the apical, middle and basal positions of the male bud for detailed analysis.

### *Morphological analyses*

The morphological traits analyzed included floral axis position and type, male bud shape, bract color, flower color and the dimensions (length and width) of both bracts and flowers. These traits were assessed using three male inflorescences per cultivar.

Bract length, bract width, flower length and flower width were measured using a vernier caliper. Observations were documented using the descriptors outlined by the International Plant Genetic Resources Institute (8). To account for positional variations along the bud, three sections were analyzed from each inflorescence.

### *Anatomical analysis*

Sections of male buds, including bracts and flowers from the apical, middle and basal positions of three inflorescences, were carefully removed and preserved in FAA50 solution (a fixation mixture of formalin, glacial acetic acid and 50 % ethanol in a 1:1:18 v/v ratio) (9).

The samples were dehydrated using 70 % ethanol and embedded in glycol methacrylate (Leica Historesin®) to prepare permanent slides. Longitudinal and transverse sections were obtained using a Leica R-2145 rotary microtome. The sections were then stained with toluidine blue for histological analysis (10, 11).

Photomicrographs were captured using an Olympus CX31 light microscope equipped with a C7070 control unit.

### *Culture Medium and explant preparation*

The MS medium was used as the primary nutrient medium. Nutrient media other than the standard inorganic salts comprised sucrose (30 g $L^{-1}$) and clarigel (0.23 %) as gelling agents.

The growth regulators used included 6-BAP (Benzyladeno-purine) @ 4 mg $L^{-1}$ during the initiation stage, which was maintained until white callus formation. For shoot formation, a reduced concentration of 2 mg $L^{-1}$ 6-BAP was used.

The male inflorescence tip was employed as an explant. It was initially washed under running tap water to remove surface contaminations and stickiness. The explant was then wiped with 70 % ethanol, followed by the manual removal of the nodal flower cluster along with the bract axil (12).

## Results and Discussion

### *Inflorescence morphology*

The inflorescence of *Musa x paradisiaca* cultivars CO 2 (AB genome) and CO 3 (ABB genome) is a mixed-branched spadix located at the distal end of the main stem axis. This inflorescence develops on a transitional, pendulous peduncle and comprises several flower clusters, each enclosed by a purple-red bract.

Each nodal cluster contains two rows of flowers arranged in layers, with approximately 18 to 20 flowers per node in both cultivars, separated by wide sub-tending bracts (Fig. 2B and 3B). These observations align with the earlier reports (6,13).

The inflorescence is divided into distinct zones: female flowers, positioned at the basal region, develop into fruits, sterile bisexual flowers occupy the middle and male flowers are located at the distal end, marking the termination of the floral axis. Throughout the growth process, the floral axis continued to extend until it fully matured. The bracts and flower clusters are spirally arranged around the main axis, creating a compact conical structure (13, 14) (Fig. 1).

### *Morphological features*

CO 2: The floral axis in CO 2 was positioned at an angle from the centre of the pseudostem, bearing neutral flowers above the male bud, while the lower stalk remained bare (Fig. 2A). The male bud was lanceolate-shaped (Fig. 2B), with a narrow ovate base, a small shoulder and a slightly pointed apex (Fig. 2C). The adaxial surface of the outermost bracts exhibited a uniform purple-brown coloration extending to the apex, while the abaxial surface was pink- purple (Fig. 2C, 2D). Strong imbrication is evident in young bracts that overlap at the apex. These bracts lifted sequentially, rolling back (revolute) before falling off (Fig. 2B).

These observations contrast with a previous research (14), which reported that the diploid bananas *Musa* AA group 'Nam Thai' and *Musa* BB group 'Ta Nee' as having male buds with obtuse bract apices, different bract lifting patterns and distinct color variations.

The flower exhibited a light purplish- cream to a yellow hue at the apex (Fig. 2B). Each flower consisted of a pink-purple compound tepal with a yellow lobe (Fig. 2F), a free oval-shaped tepal with pink-tinted corrugations (Fig. 2G), a cream-colored straight style, inserted and arched ovary (Fig. 2H) and five stamens with cream filaments and large anthers (Fig. 3).

The morphological dimensions of the bracts (Fig. 2D) and flowers (Fig. 2E), along with the number of flowers per nodal cluster in cultivar CO2, are summarized in (Table 1).

CO 3: The floral axis was vertically aligned with the pseudostem and remained bare above the male bud, lacking neutral flowers (Fig. 3A).

The male bud was ovoid in shape (Fig. 3B, 3C). The bracts were ovate, with a medium shoulder base and a pointed apex (Fig. 3D), differing from the obtuse apex described for the triploid (ABB) cultivar Thep Panom banana (14). The adaxial surface of the bracts exhibited a uniform purple-brown coloration extending to the apex, while the abaxial surface was bright crimson red, with coloration fading towards the base (Fig. 3D, 3C). Slight imbrication was observed, with minimal overlap of young bracts at the apex. Like CO 2, the bracts in CO 3 lifted sequentially, rolling back before detaching (Fig. 3D, 3E).

The flowers were light purple with yellow highlights at the apex (Fig. 3B), differing from the pale pink flowers reported for triploid Thep Panom bananas (14, 15). Each flowers had a pink- purple tepal with a yellow lobe (Fig. 3F), a translucent white, oval-shaped free tepal with a slightly developed thread-like apex (Fig. 3G), well-developed stamens with cream filaments and yellow anthers (Fig. 3I) and a gynoecium with a white style, curved base, yellow stigma and straight ovary (Fig. 3H).

The morphological dimensions of the bracts (Fig. 3C) and flowers (Fig. 3E), along with the number of flowers per nodal cluster in CO 3, are summarized in (Table 1).

The number of flowers, bracts and flower dimensions were larger in CO 3 compared to CO 2. This variation may be attributed to floral axis orientation, vascular bundle efficiency and cultivation adaptation. The vertical floral axis orientation of CO 3 allows better spatial arrangement and enhanced nutrient distribution, supporting a higher number of flowers per node. In contrast, the angled floral axis of CO 2 may limit flower development due to less efficient nutrient flow and structural constraints, which aligns with the results of a previous study (13). Flower morphology plays an important role in determining the success of tissue culture (16).

### *Size and compactness and tissue composition*

Compact structures with smaller flowers are more manageable and easier to sterilize during tissue culture. Their compact arrangement minimizes the surface area exposed to potential contaminants, thereby improving culture success rates. In contrast, larger flowers pose challenges during handling, sterilization and culture establishment due to their increased surface area and potential mechanical damage.

Compact flowers often contain higher concentrations of meristematic cells, which actively divide and are essential

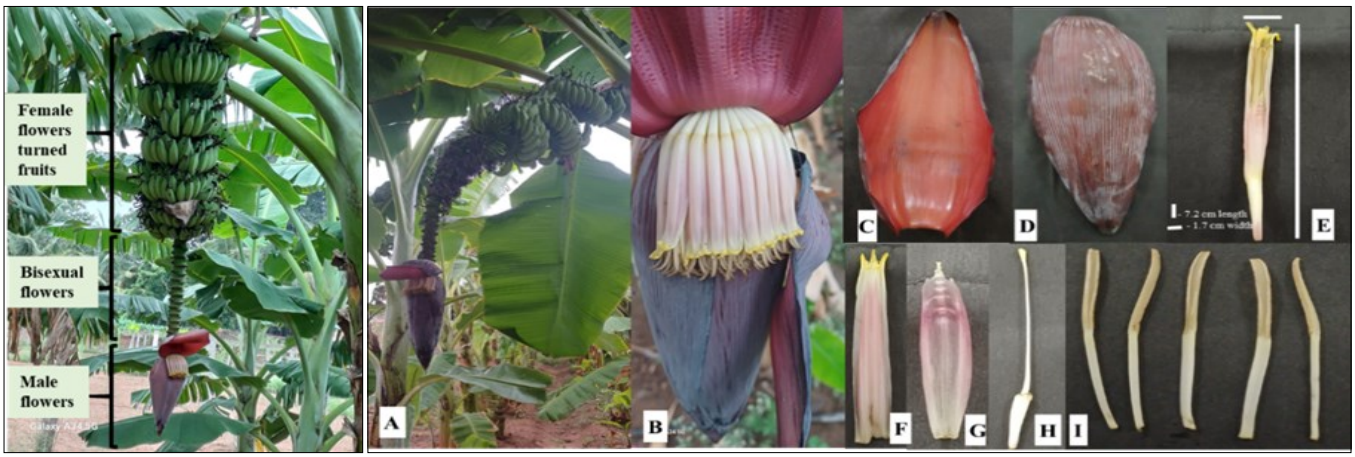


**Fig. 1.** Complete inflorescence with female, bisexual (empty nodes) and male bud with flowers.

**Fig. 2.** (CO 2) (A) Floral axis type at an angle, (B) Male bud with flower cluster, (C) Abaxial side of bract, (D) Adaxial side of bract, (E) Single flower, (F) Compound tepal, (G) Free tepal, (H) Gynoecium (I) Androecium.

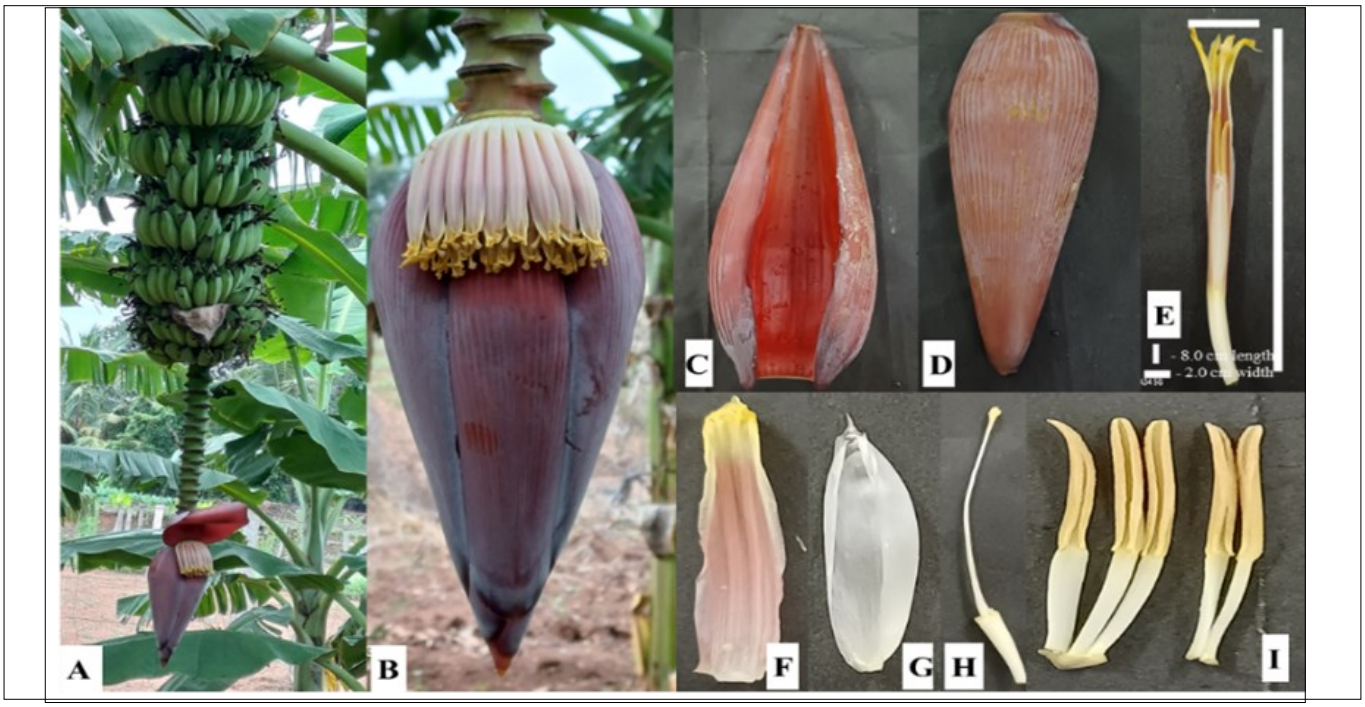


**Fig. 3.** (CO 3) (A) Vertical floral axis type, (B) Male bud with flower cluster, (C) Abaxial side of bract, (D) Adaxial side of bract, (E) Single flower, (F) Compound tepal, (G) Free Tepal, (H) Gynoecium (I) Androecium.

**Table 1.** Quantified morphological dimensions

| Morphological character | Cultivar. CO 2 | | | Cultivar. CO 3 | | |
|---|---|---|---|---|---|---|
| | Apical | Middle | Basal | Apical | Middle | Basal |
| **Bract Length (cm)** | 1-5 | 6-9 | 15-17 | 2-5 | 7-10 | 17-20 |
| **Bract Width (cm)** | 2-3 | 4-7 | 9-10 | 2-4 | 5-8 | 12-15 |
| **Flower Length (cm)** | 0.5-4 | 4-6 | 7.2-7.5 | 0.5-5 | 5-8 | 7.5-8.0 |
| **Flower Width (cm)** | - | - | 1.5-1.9 | - | - | 1.7-2.0 |
| **Number of flowers** | 14-18 | | | 18-20 | | |

for initiating callus formation and subsequent organogenesis. These findings are supported by previous studies (17, 18).

While larger flowers typically possess well-developed vascular tissues that enhances nutrient flow efficiency this advantage may be counterbalanced by their higher metabolic demand (19). Additionally, a study explored the correlation between flower size, vascular bundle efficiency and nutrient transport during tissue culture (15).

*Surface morphology:* The presence of specialized structures, such as papillae in bracts, was observed in CO 2 but was absent in CO 3. These structures enhance water retention and nutrient absorption. These features improve the ability of explants to establish themselves in culture and resist desiccation. The role of papillae in water retention and nutrient absorption, contributing to explant establishment *in vitro*, has been previously highlighted in the study (11).

*Contamination risk:* Larger and more open floral structures may increase the risk of microbial contamination due to greater exposure to external pathogens. In contrast, compact flowers and robust bract imbrication serves as physical barriers, effectively reducing the likelihood of contamination (20) explained that compact floral structures and bract imbrication play a crucial role in minimizing contamination risks during tissue culture.

*Reproductive tissue characteristics:* The absence of ovules in both cultivars reduces reproductive interference during tissue culture. However, anatomical differences in the stamen and tepal structures may influence dedifferentiation and callus initiation. These present suggestions are consistent with the discussion on the absence of ovules and their impact on minimizing contamination and reproductive interference during tissue culture (21).

### *Anatomical features*

The anatomical features of the bracts showed no significant differences between the CO 2 and CO 3 cultivars, except for the presence of papillae. The surface view of the outermost bracts revealed straight, thin anticlinal walls on both the adaxial and abaxial epidermis (Fig. 4A, 5A). In cross-section, both cultivars exhibited a uniseriate epidermis composed of polygonal cells with thick anticlinal walls. However, spherical-to-pyriform projections on the external periclinal wall, known as papillae, were observed only on the abaxial surface of CO 2 (Fig. 4C). Tetracytic stomata with thicker walls were interspersed among common epidermal cells in both cultivars (Fig. 4C, 5C, 5H).

In CO 3, papillae were absent; however, in CO 2, the presence of papillae enhanced water retention and nutrient absorption, potentially contributing to improved explant viability. This feature is consistent with previous findings in certain *Musa* spp. cultivars (11). The presence or absence of papillae can serve as a distinguishing feature among different *Musa* genome groups. For example, cultivars in the AAB genome group, such as cv. Terra lacked papillae on the abaxial epidermis, whereas those in the AAA group did. Similar papillose structures have been documented in the epidermis of *Musa acuminata* Colla (15).

Both cultivars demonstrated collateral vascular bundles distributed throughout the mesophyll, containing well-developed xylem, fiber and phloem, which ensure efficient nutrient transport and structural integrity, making both cultivars resilient to micropropagation (Fig. 4B). These characteristics were documented in *M. acuminata* (22, 18).

The mesophyll is homogeneous and consists of multiple layers of parenchymal tissue. Smaller parenchyma cells were located near the epidermis, while larger cells occupied the centre, reflecting tissue maturity and reduced meristematic activity (Fig. 4C).

Thick-walled sclerenchymatic fibre cells, exhibiting autofluorescence, contribute to structural strength, aiding in plant erectness (Fig. 5B, 5G). Aeration chambers with irregularly shaped branching parenchyma cells and significant intercellular gaps were also observed (Fig. 5A, E, F). The inner bract, particularly in the apical region, contained a higher density of actively dividing meristematic cells, indicating greater regenerative potential compared to the outermost bracts in micropropagation. Additionally, the vascular bundles in the inner bracts were more concentrated, supporting rapid growth and development and exhibited a lighter coloration than the outer bracts (Fig. 4D, 5D). These findings are supported by research (20).

The stamens exhibit connective tissue with a thicker epidermis and multiple layers of parenchyma cells of varying shapes and sizes, characterized by thin walls and a single collateral vascular bundle within the connective region (Fig. 6B). This vascular bundle ensures nutrient transport and cellular differentiation during *in vitro* culture (17). The anthers were tetrasporangiate, with locules separated by a septum (Fig. 6A, 7A). The pollen sac area had a uniform epidermis composed of round cells, an endothecium with thickening rings and collapsed inner layers. Occasionally, spherical pollen grains were observed in the locules (Fig. 7B), indicating a capacity for cellular differentiation, which is a critical factor for successful *in vitro* regeneration, inclines with the insights of prior investigations (18).

As described previously (23), the male flower is zygomorphic (Fig. 8A, 9A) and comprises five stamens, a compound tepal and a free tepal. The free tepal features a homogeneous mesophyll, a unilayered epidermis and subepidermal layers (Fig. 8C, 9C). In contrast, the outer compound tepal has a multilayered epidermis, a homogeneous mesophyll, parenchyma and collateral vascular bundles located closer to the abaxial surface, which are well-aligned and uniform in size (Fig. 8D, 9D). The structural characteristics of both compound and free tepal contribute to mechanical resistance during handling and culture establishment, findings that are consistent with earlier results (20).

The ovary is inferior and trilocular, with unilayered external and internal epidermis (Fig. 8B, 9B) reflecting the structural stability necessary for tissue culture manipulations.

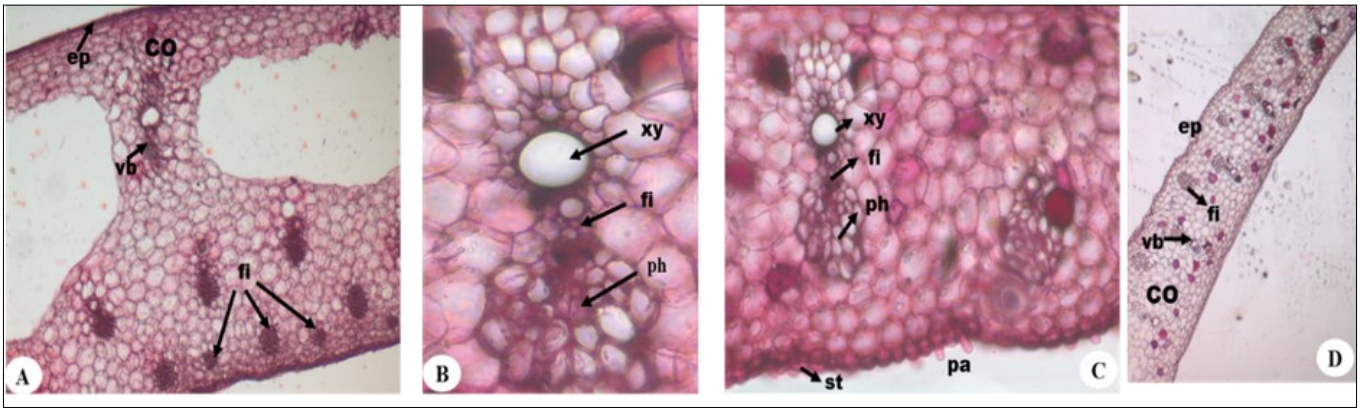


**Fig. 4.** (CO 2) Photomicrographs of cv. CO 2 bract anatomy outer (A-C) and inner (D) Co, collenchyma; ep, epidermis; fi, fiber; pa, papillae; ph, phloem; st, stomata; vb, vascular bundles; xy, xylem.

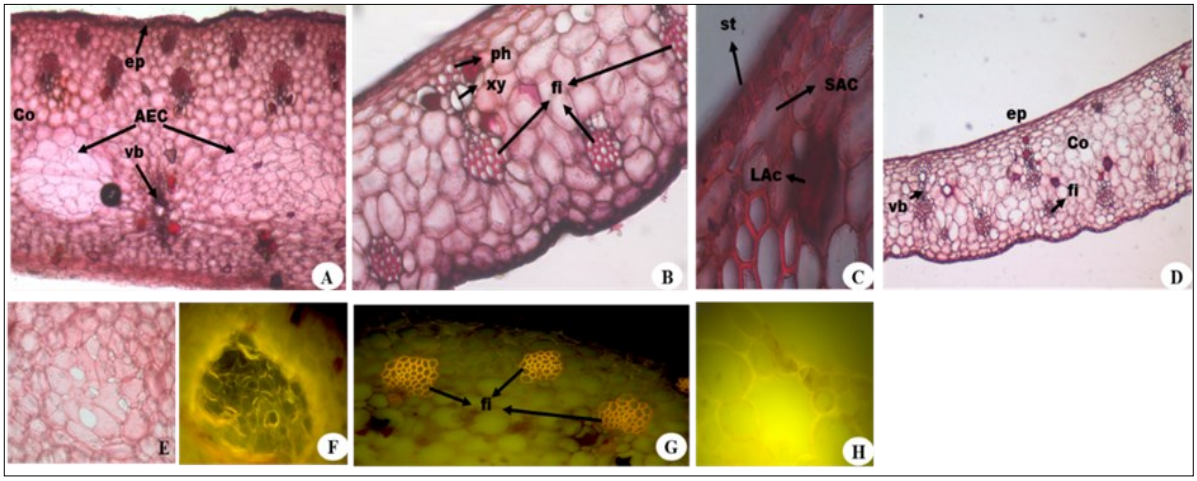


**Fig. 5.** (CO 3) Photomicrographs of cv. CO 3 bract anatomy outer (A-C) and inner (D) Co-collenchyma; ep-epidermis; vb-vascular bundle; (E, F) AEC-Aeration chambers and their irregular cells; (G) fi-fiber; ph-phloem; xy-xylem; (C,H) st-stomata; SAC-Stomata air chamber; LAc-Latex cell. (F,G,H – are auto fluorescent cells).

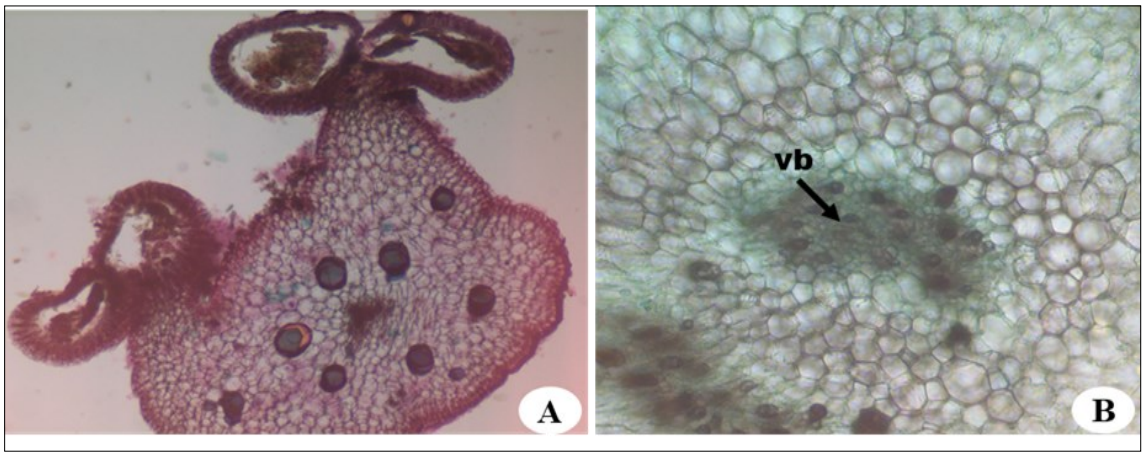


**Fig. 6.** (CO 2) Photomicrographs of cv. CO2 anther anatomy - transverse section. (A) Tetrasporangiate anther (B) Connective region vb-vascular bundle.

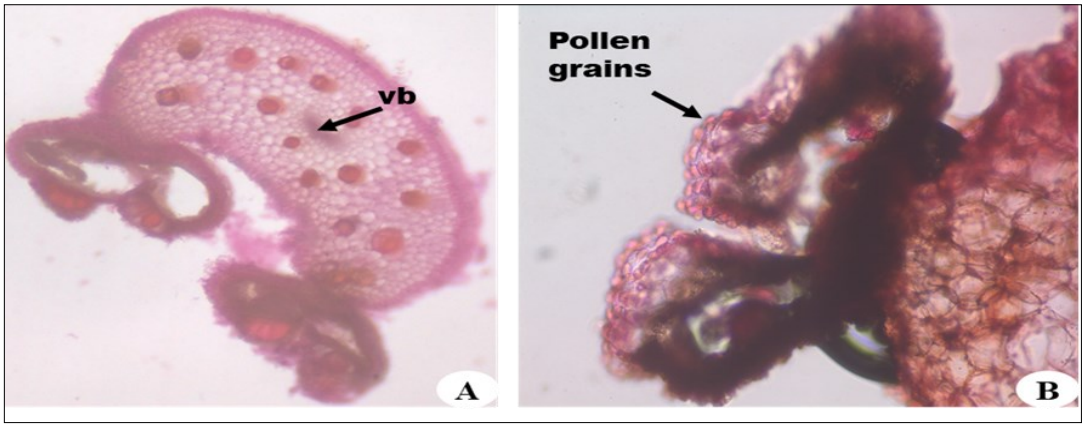


**Fig. 7.** (CO 3) Photomicrographs of cv. CO3 anther anatomy - transverse section. (A) Tetrasporangiate anther with connective region, vb-vascular bundle. (B) Spherical Pollen grains in locules.

These observations are consistent with previous findings on the genus *Musa* (21, 17). Cross-sections of the middle of the corolla tube (Fig. 8E, 9E, 9F) reveal a uniseriate epidermis on both sides, consisting of circular to elliptical ordinary cells and mesophyll with multiple layers of parenchyma cells. Notably, no ovules were observed in the analyzed samples, indicating a non-functional gynoecium. The absence of reproductive activity reduces the risk of contamination, thereby simplifying the tissue culture process. The male inflorescence tips of banana cultivars exhibit significant potential as explants for tissue culture because of their unique morphoanatomical characteristics. Idioblasts containing calcium oxalate (CaOx) crystals were identified in the anther region (Fig. 10A) and within the ovary in the form of raphides (Fig. 10B, 10C), embedded in parenchyma cells in both cultivars. The presence of CaOx crystals influences cell differentiation and tissue development, thereby promoting the formation of organized structures during *in-vitro* culture. These findings align with former studies (18), which reported the presence of numerous CaOx crystals and raphides in the pollen grains of *Tinantia anomala*. Their study highlighted the role of these structures in cellular organization and tissue stability during culture. Additionally, the presence of CaOx crystals serves as a natural defence mechanism against insect pests, reducing the likelihood of contamination when these tissues are used as explants in tissue cultures (22-24).

### *Response of male inflorescence tips as an explant*

The male inflorescence tips (Fig. 11A, 11B) showed a strong

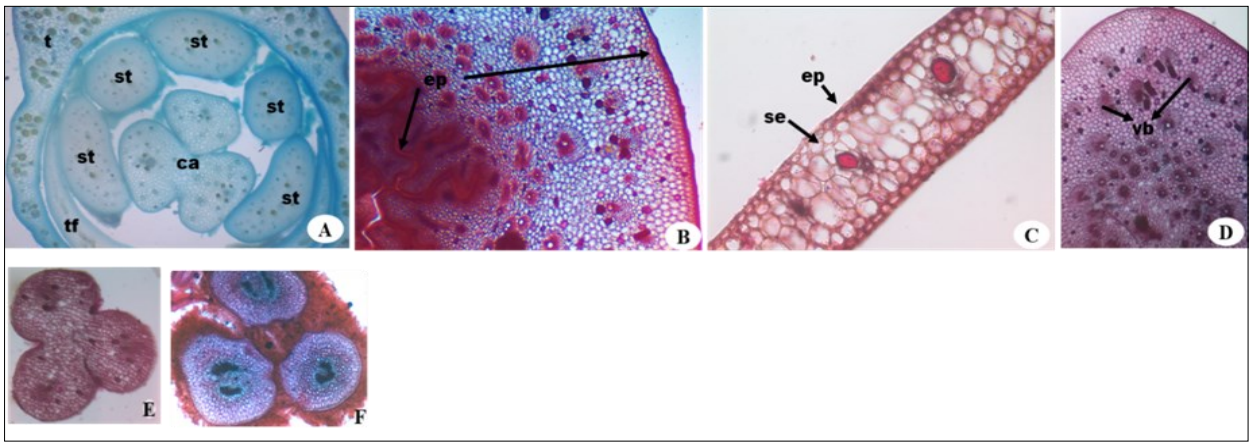


**Fig. 8.** (CO 2) Photomicrographs of cv. CO 2 flowers and ovary anatomy – transverse sections. (A) Carpel five stamens, free tepal and outer tepals. (B) Ovary (C) Internal tepal (D) External tepal. (E,F)-trilocular ovary, Ca- carpel; ep-epidermis, se- subepidermal layer; st-stamen; t-tepal; tf-free tepal; vb-vascular bundle.


**Fig. 9.** (CO 3) Photomicrographs of cv. CO 3 flowers and ovary anatomy – transverse sections. (A) Carpel, five stamens, free tepal and outer tepals. (B) Ovary (C) Internal tepal (D) External tepal, (E, F)-trilocular ovary. Ca- carpel; ep-epidermis, se- subepidermal layer; st-stamen; t-tepal; tf-free tepal; vb-vascular bundle.

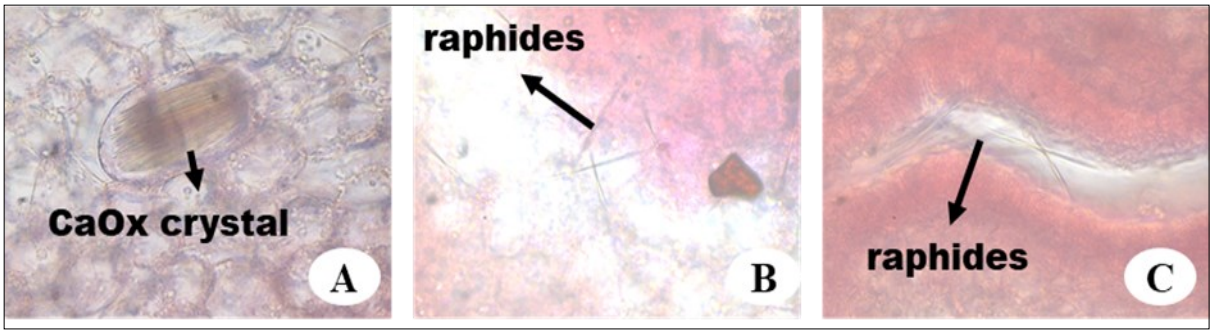


**Fig. 10.** Photomicrographs of anther and ovary showing CaOx crystals (A) and raphides (B, C).

response to tissue culture media, with early greening and swelling. CO 2 explants outperformed CO 3, exhibiting faster callus formation and shoot regeneration, due to the presence of papillae that enhance water retention and nutrient absorption. The compact floral structure and smaller flowers in CO 2 facilitated handling and sterilization, reducing contamination risk, whereas CO 3's larger flowers, despite better spatial arrangement from its vertical floral axis, posed challenges. Although in CO 2 the angled floral axis may have slightly restricted nutrient flow, its higher meristematic cell density in inner bracts supported better regeneration. Both cultivars lacked ovules, minimizing contamination risks, but the absence of papillae in CO 3 may have reduced its viability. These responses suggest efficient absorption and transport of nutrients, likely attributable to anatomical features discussed (7). The explant response to different media compositions is summarized in Table 2.

Callus formation (Fig. 11D) occurred as lignin-rich cell walls weakened, enabling dedifferentiation and organogenesis, as observed that the reduction in lignin density facilitates cell dedifferentiation and subsequent differentiation and organogenesis (18). A successful shoot formation (Fig. 11E) was followed highlighting the ability of male inflorescence tips to regenerate into complete plantlets which is consistent with findings on banana organogenesis (4). The contamination rate was notably low (Fig. 11F, 11G), likely due to the absence of ovules, further supporting the suitability of male inflorescence tips for tissue culture consistent with the earlier observations (17).

Male inflorescence tips offer a sustainable alternative to conventional explants like sucker shoots and rhizomes, being non-destructive, readily available and cost-effective. Their high meristematic cell density enhances regeneration, while CO 2's compact structure and papillae improve efficiency over CO 3. However, challenges such as larger flower size in CO 3, thick cell wall delaying callus formation and sensitivity to handling can be addressed with optimized sterilization, lignin-reducing treatments and tailored media compositions. These findings highlight the potential of male inflorescence tips for efficient banana micropropagation, reducing costs and resource use, with applications for other varieties or crops with similar anatomical traits, in line with (20, 25, 21, 4).

## Conclusion

**Table 2.** Explant response

| Concentration of 6-BAP in MS medium | Explant response | Duration (Mean values) |
|---|---|---|
| 4 mg $L^{-1}$ | Opening up and Greening | 16.2 days |
| | Swelling and bulging | 28.4 days |
| | Callus (white buds) formation | 122.2 |
| | Number of buds formed | 2-6 |
| 2 mg $L^{-1}$ | Number of shoots formed | 12-15 per nodal cluster |

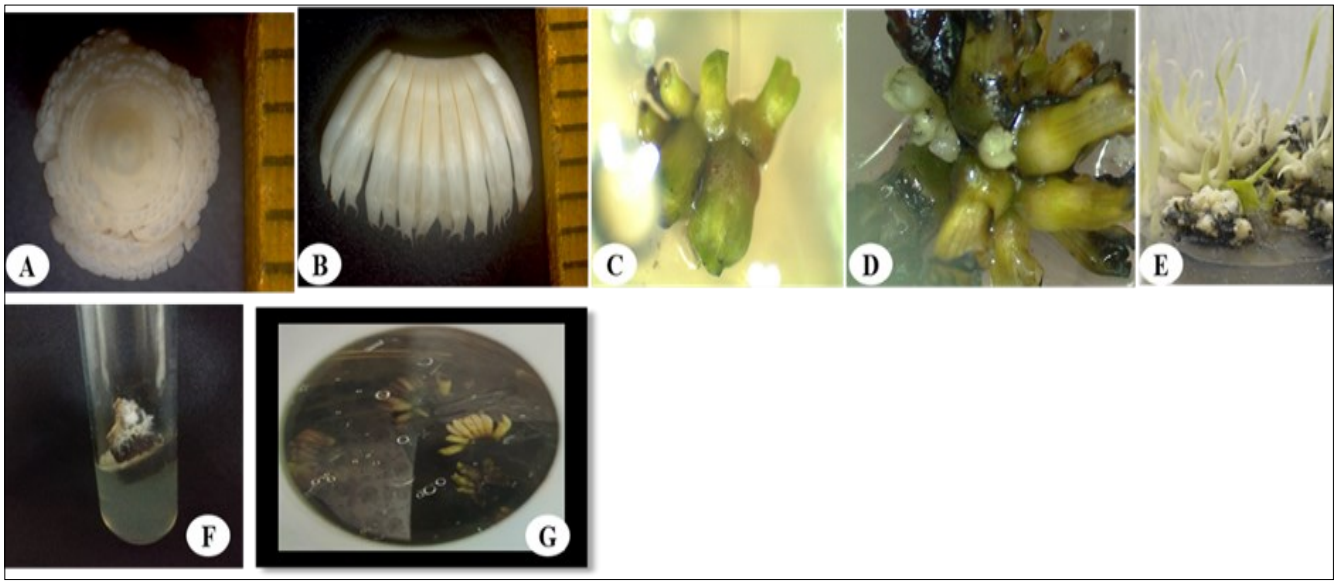


**Fig. 11. (CO 2)** (A-B) Male inflorescence tips, (C) Initial Greening, (D) Formation of callus, (E) Shoot formation, (F-G) Contamination effects.

The study revealed that cultivars CO 2 and CO 3 show significant differences in floral morphology and anatomy, which influence their effectiveness as explants in tissue culture. Key morphological differences include the position and type of the floral axis, male bud shape, bract shape and colour and number of flowers, with CO 3 producing more flowers per cluster than CO 2.

CO 2 exhibits a more compact structure and is anatomically distinguished by the presence of papillae on the abaxial surface of its bracts, which are absent in CO 3. However, both cultivars share zygomorphic floral structure and the presence of calcium oxalate (CaOx) crystals and raphides.

Overall, the unique morphoanatomical features of the male inflorescence tips in both cultivars make them highly suitable for micropropagation. These traits ensure efficient growth and development while minimizing contamination risks under tissue culture conditions, thereby their potential for reliable banana propagation. These findings pave the way for optimizing tissue culture protocols for banana propagation, with potential applications in other plant species exhibiting similar morphoanatomical features.

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## Authors' contributions

SS, MI conceived of the study and participated in its design and coordination. MNB, KC, SA participated in data analysis and drafted the manuscript. SS conceived the original and supervised the project. All authors read and approved the final manuscript

## Compliance with Ethical Standards

**Conflict of interest:** Authors do not have any conflict of interests to declare.

**Ethical issues:** None

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