Development of Competent Multiple Meristem Cultures from Immature Male Bud of Banana, CO 2 (AB) and CO 3 (ABB)

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Abstract

A protocol was developed for the establishment of multiple meristem cultures from immature male buds of banana hybrids CO 2 and CO 3 using two explant approaches: floral hands with bract axil and longitudinally sectioned floral apex segments. Among the treatments, longitudinal sectioned segments demonstrated a superior morphogenic response, with consistent 100% initiation across both hybrids. The earliest formation of shoot-forming nodules (SFNs) was observed under treatment T_9 (6 mg/l BAP + 1 mg/l NAA), occurring at 21.2 days (CO 2) and 21.3 days (CO 3), followed by the development of white compact meristems (WCMs). A strong positive correlation was noted between SFNs and WCMs with the highest WCMs (9.0 in CO 2; 9.5 in CO 3) resulting from 6.0 SFNs per segment.

Shoot induction was significantly enhanced in S_4 (3 mg/l BAP + 2 mg/l Kinetin), producing 14.5 shoots in CO 2 and 15.1 in CO 3. Longitudinal sectioned explants produced up to 45% more WCMs and 35% more shoots compared to floral hands, demonstrating their higher responsiveness and regeneration efficiency. The study demonstrates the potential of male floral explants, particularly longitudinal segments, for high-frequency propagation through multiple meristem cultures, offering a scalable solution for the commercial production of uniform, high-quality banana planting material.

Keywords: Banana, immature male bud, *in vitro*, multiple meristems.

Introduction

Banana (*Musa* spp.) is known as the "Kalpavriksha, the divine tree" as every part of the plant holds economic and nutritional value[5]. It is one of the most traded tropical fruits worldwide, serving as a staple food for millions and significantly contributing to food security, employment and rural livelihoods[3]. India leads global banana production, accounting for nearly 26.5% of the total global output, with major cultivation in States like Tamil Nadu, Maharashtra, Gujarat and Andhra Pradesh[10]. *In vitro* banana plantlets possess high export potential, with India being a leading global exporter of banana microplantlets. The high yield and low production costs contribute to the economic importance of banana cultivation[18].

However, the traditional propagation of bananas through suckers is slow, resulting in uneven growth and yield[13]. Micropropagation offers a reliable solution to tackle these challenges, through *in vitro* shoot-tip culture for rapid multiplication of disease-free, genetically uniform planting material year-round, even though it produces a limited number of plantlets per cycle[5,16]. The clonal nature of banana production further exacerbates this limitation, its susceptibility to pests and diseases, resulting in diminished genetic diversity. Additionally, this method often faces challenges due to lengthy sterilization processes and significant phenolic exudation, which can negatively impact the establishment and growth of cultures.

One of the unique attributes of Kalpavriksha is its remarkable ability to regenerate from various tissues such as suckers, rhizomes, shoot-tips[23], leaf sheaths, male inflorescence[12], floral apices[21] and female flowers[20]. Among all the explants, immature male floral buds have shown promise, because the explant collection can be done without causing any damage to the mother plant, also due to their rich meristematic zones and responsiveness to plant growth regulators. While floral hands with bract axils have been used in earlier protocols, their response is often variable and restricted by internal tissue access.

In contrast, longitudinally sectioned floral apex segments expose deeper meristematic layers, potentially improving nutrient absorption and hormonal sensitivity, thereby enhancing morphogenic response. Among various tissue culture techniques, multiple meristem cultures (MMC) is meristematic domes (cauliflower-like bodies), also known as shoot-forming nodules (SFNs), scalps, multiple bud clumps or proto-corm-like bodies from a single explant.

It is significant due to their high proliferating, regeneration efficiency and ability to maintain genetic fidelity[22,25], reducing the dependency on limited sucker availability and are ideal for commercial propagation. Growth regulators such as 6-Benzylaminopurine (BAP), a synthetic cytokinin,

stimulate shoot proliferation and cell division when combined with auxin[9].

This study aims to develop competent multiple meristem cultures from the male inflorescence of banana hybrids (Coimbatore 2) CO 2 (AB) and (Coimbatore 3) CO 3 (ABB) to optimize the protocol to enhance propagation efficiency and to ensure sustainable large-scale production with high-quality planting material.

Material and Methods

Plant Materials: The immature male buds of *Musa paradisiaca* L. (Musaceae) hybrids CO 2 and CO 3 (Fig. 1A) were collected three months before harvesting from the orchard, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, India (11.0096° N, 76.9294° E). The male inflorescences were cultured within three days of collection.

Preparation of Explants: The outer bracts of collected immature male buds are manually removed till the white inner bracts are visible. This trimmed explant is taken into the laminar airflow chamber swabbed with 70 per cent ethanol and further shortened till the male bud reaches about 4-6 cm in size (Fig. 1B). This 4-6 cm trimmed floral meristem explant was used in two ways:

1. Carefully extract the floral hands with bract axil intact (Fig. 1C); tiny bracts and floral hands were aseptically removed without damaging the apical dome and floral hand.
2. Floral meristem longitudinally sectioned into smaller segments (3-4 cm) with the central floral axis was exposed (Fig. 1D), prepared by bisecting and sub-sectioning the bud to expose multiple meristematic sites for culture initiation.

Media Preparation: Both the explants were initiated in MS medium[17] supplemented with 4% sucrose (w/v), 0.5% ascorbic acid (v/v), 0.25% agar (w/v), adjusted to pH 5.8 and autoclaved at 121°C for 15 minutes. The cultures were incubated at 25 ± 2˚C under cool white fluorescent lamps with 16 hrs photoperiod and a light intensity of 1100 lux. The fresh media comprised of different hormonal concentrations: 6-BAP (T_1: 4 mg/l, T_2: 5 mg/l, T_3: 6 mg/l) and combinations of 6-BAP/NAA (T_4: 4/0.5 mg/l, T_5: 4/1 mg/l, T_6: 5/0.5 mg/l, T_7: 5/1 mg/l, T_8: 6/0.5 mg/l, T_9: 6/1 mg/l) for the explants to produce multiple compact meristems.

Shooting: Once the White Compact meristem (WCM) was induced, they were transferred to induce shoots after two subcultures. The shooting medium comprises of the combinations of 6-BAP/Kinetin; S_1: 2/1.5 mg/l, S_2: 2/2 mg/l, S_3: 3/1.5 mg/l, S_4 : 3/2 mg/l. The explants were subcultured every 4 weeks by using the same media to mitigate the explant browning problem. The well-elongated shoots were transferred to the rooting medium comprising of IBA @ 2 mg/l.

Growth observation: The growth of both the explants was observed at the initiation stage by using the following parameters: Percentage of explants responding (4 weeks after inoculation), Number of days taken for white compact meristem (WCM) to be formed (@ 21 days intervals), Number of White compact meristem (WCM) formed (1st subculture), Number of days taken for shoot-forming nodules (SFNs) to be formed (@ 21 days intervals), Number of shoot forming nodules formed (1st subculture) and Number of shoots (4 weeks after inoculation in shooting media).

Results and Discussion

The response of both the explants to initiation was evaluated after four weeks, which showed significant differences between the two types of explants and the cultivars. When the floral hands with intact bract axils are used as an explant, CO 2 showed response percentages between 96–100% while CO 3 registered a slightly lower response, ranging from 92–100%. The different reactions of cultivars to the floral hand method indicate that the plant's genetic makeup interacts with how the explant is prepared. This aligns with the results of studies by Gubbuk et al[8].

In contrast, longitudinally dissected segments consistently exhibited a 100% response in both cultivars across multiple treatments (Fig. 2). The observations of enhanced and consistent responsiveness of longitudinally dissected segments, which expose a greater extent of meristematic tissue, likely offer a more reliable and uniform response compared to the floral hand explants. These findings align with research indicating higher regeneration frequencies when explants are processed to maximize meristematic exposure[26].

Male floral hands with bract axil intact explants: The floral hands of different sizes (Fig. 3a) were initiated in a way with the bract axil immersed in the medium (Fig. 3b). After 21 days, the explant turned green, the bract axil expanded and each finger swelled and bulged making the curved explant stand straight and exposing the inner basal part of the floral hand irrespective of different treatments (Fig. 3c). Similar observations were made by Wirakarnain[27] in his study; the floral hands swelled up and turned green in colour, with the bract turning outward. The time required and the number of white compact floral meristems formed varied significantly across treatments and varieties.

The white compact floral meristems were initiated within 122-126 days in CO 2 and 120-125 days in CO 3, with T_9, T_8 and T_6 being the earliest and T_1 and T_4 taking the longest duration for initiation in both varieties (Table 1). The present study results indicate an enhanced early morphogenic response, which are not in line with Lusiyanto et al[14], who used complete male flower apices as explants and found that combining high cytokinin (BAP) and low auxin (IAA) in the initiation media led to callus development rather than nodule or shoot formation.

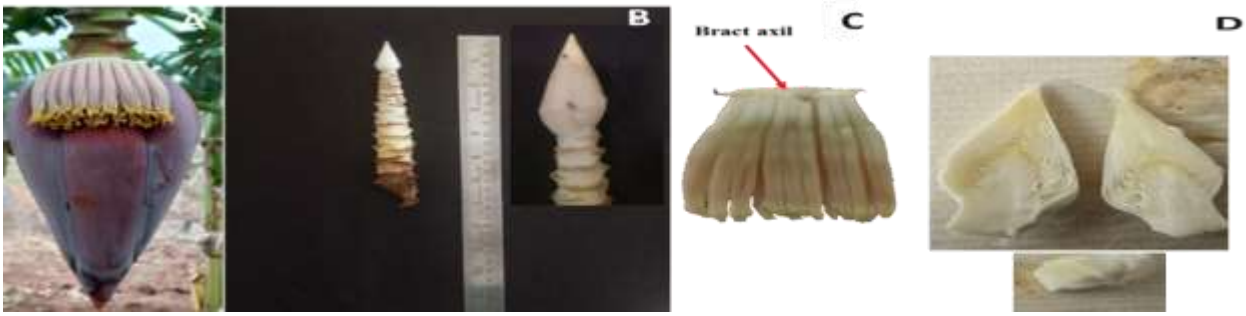

Fig. 1: (A) Immature male bud of CO 2 and CO 3 banana cultivars collected from the Tamil Nadu Agricultural University orchard, (B) Trimmed male bud, (C) Floral hands with bract axil intact and (D) Longitudinally slit male bud with floral axis.

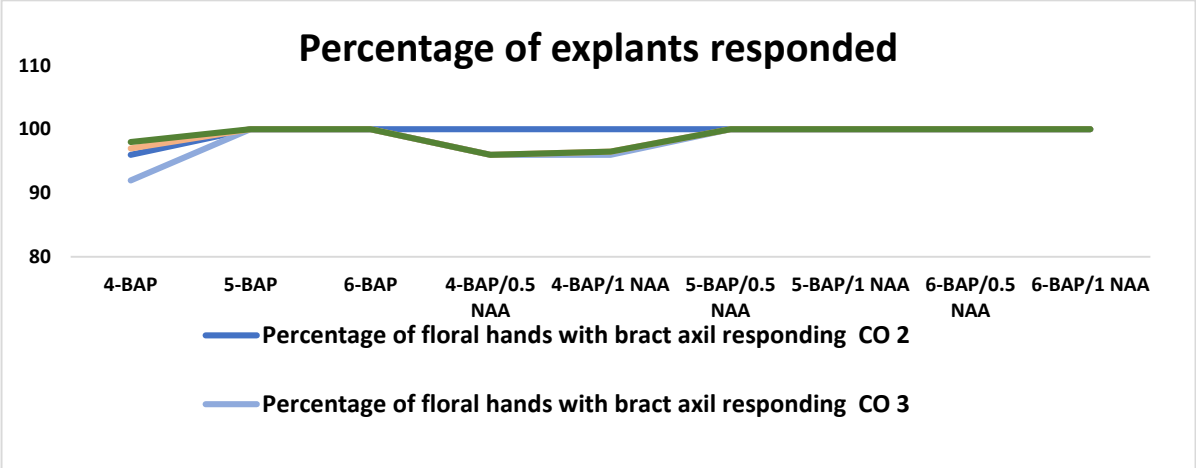

Fig. 2: Percentage of response of floral hands with bract axil and longitudinal male bud apex segments

A maximum number of WCM (Fig. 3d) of 8.0 in CO 2 and 6.5 in CO 3 was recorded in T_9. In both cultivars, T_9 was followed by the treatment involving higher BAP concentrations combined with NAA, T_8 (6.5 and 5.2) and T_3 (5.7 and 2.8) respectively. At lower cytokinin concentrations, both cultivars showed minimal WCM, 1.5 in CO 2 and 1.2 in CO 3 (Table 1), but as the concentration increased and auxin combinations were optimized, the number of WCMs increased proportionally, confirming the synergistic role of cytokinins and auxins in promoting meristematic proliferation.

The formation of compact nodular meristems aligns with previous reports suggesting that high cytokinin levels reduce apical dominance, facilitating the proliferation of multiple shoot initials from exposed meristematic tissues[15,24]. Moreover, the enhanced response of CO 2 may be attributed to its genotypic sensitivity to cytokinin-induced signalling, a trait often genotype-dependent in Musa spp[2].

The meristems after the second subculture were transferred into the shooting medium (Fig. 3e). Among the four treatments, S_4 (3 mg/l BAP + 2 mg/l Kinetin) resulted in the highest shoot proliferation, with 12.3 shoots per explant in CO 2 and 14.3 shoots in CO 3 (Fig. 3f). This was followed by S_3 (3/1.5 mg/l), which showed a strong proliferative response with 11.6 and 12 shoots in CO 2 and CO 3 respectively.

The lowest shoot numbers were observed in S_1 (2/1.5 mg/l), producing 7.0 shoots in CO 2 and 8.5 in CO 3 (Table 1). These findings are supported by previous studies that demonstrate how BAP enhances meristematic activity, while kinetin promotes cell division and elongation, especially when used in combination[1]. Furthermore, the increasing response at 3 mg/l BAP suggests that higher cytokinin concentrations help to overcome apical dominance, thereby allowing multiple shoot initiation from a single explant [(24)]. The elongated shoots after 6 weeks were transferred to the rooting media (Fig. 3g).

Longitudinally sectioned floral apex segment explants: The explant network (Fig. 4b) is the interconnected group of cells and tissues which develop from the original explant-longitudinal section segments (Fig. 4a). This network gives rise to various structures including calluses, shoot-forming nodules. Endogenous auxin activity, which is seen in meristematic cells, causes nodules or swelling explants[19]. Nodules begin on the meristematic side next to the bract attachment (flower base)[6]. In both the hybrids CO 2 and CO 3, explants formed visible shoot forming nodules (Fig. 4c) significantly earlier than floral hand explants. The earliest SFN initiation occurred under treatment T_9, at 21.2 days in CO 2 and 21.3 days in CO 3. These results align with Kalimuthu et al[11], who reported early induction when higher BAP levels were applied during the early culture phase.

These nodules regenerate into white compact meristems after 50-60 days of initiation and some of the nodules grow into shoots directly from the network without the formation of meristems (Fig. 4d). Alternatively, shoots can arise are from white compact meristems that are formed within the explant network. An increase in the number of SFNs was consistently associated with a higher number of WCMs in both cultivars. The highest SFNs produced among the explant network were recorded as 6.0 in CO 2 and CO 3, which corresponded to the maximum WCM formation of 9.0 in CO 2 and 9.5 in CO 3, respectively (Table 2).

The hybrid CO 3 exhibited marginally higher SFN and WCMs in later treatments, ranging from 2.2 to 6.0 SFNs and 5.1 to 9.5 WCMs while CO 2 ranged from 2.0 to 6.0 SFNs and 4.5 to 9.0 WCMs respectively. These results suggest a slightly enhanced responsiveness of CO 3 in converting nodules into meristems under optimized hormonal conditions. Notably, at lower SFN (2–3 nodules), WCM development remained modest (around 4.5–5.4), while treatments with >5 nodules consistently resulted in WCMs above 6.5, further confirming the functional link between SFN density and shoot regeneration potential.

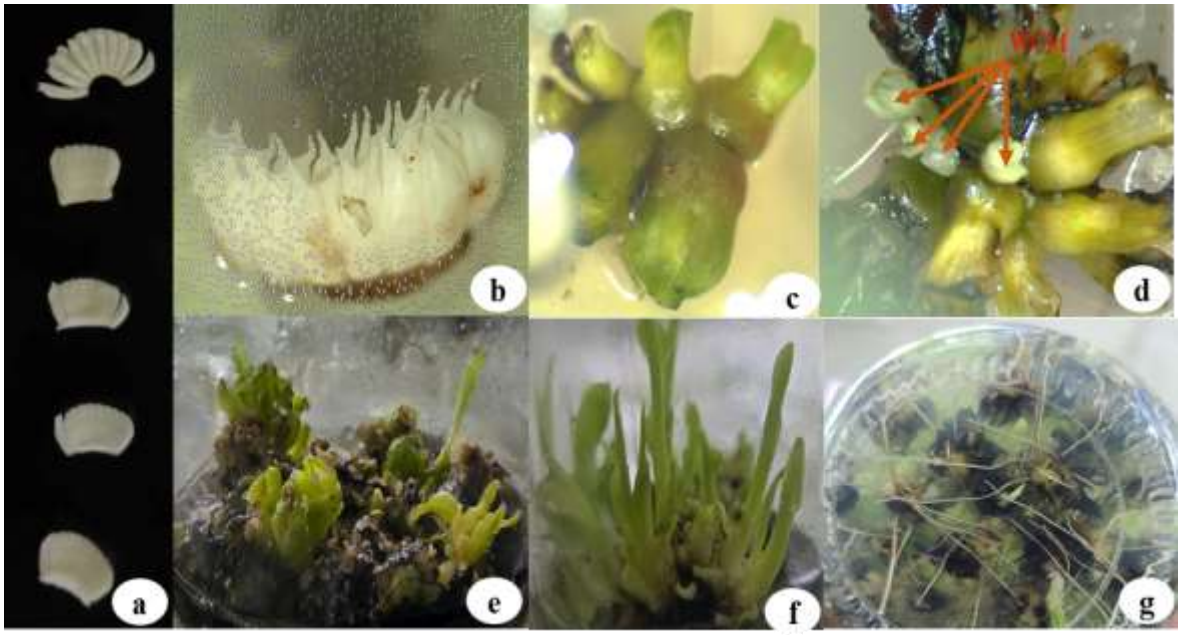

Fig. 3: (a) Floral Hands of different sizes, (b) Initiation of Floral Hand with the bract axil intact, (c) Green and Swollen Floral Hand, (d) Initiation of White Compact meristems, (e) Shoot initials from the meristem, (f) Shoots at the second subculture and (g) Rooting of the elongated shoots.

Table 1
Effect of Cytokinin and Auxin Combinations on the initiation of White Compact Meristems from Floral hands with bract axil in Banana hybrids CO 2 and CO 3

Treatments	**Number of days taken for the White Compact Meristem to be formed**		**Number of White Compact Meristems**	
Initiation	**CO 2**	**CO 3**	**CO 2**	**CO 3**
T_1	126.2	125.4	1.5^e	1.2^e
T_2	125.6	124.6	2.5^d	2^e
T_3	122.5	120.8	5.7^b	5.8b
T_4	125.4	125	2^e	2.5^d
T_5	125.2	124.8	2.3^d	2.5^d
T_6	125.2	123.6	3.5^c	3.8^c
T_7	122.4	123.4	4^c	4.5^c
T_8	122.6	120.6	6.5^b	5.2^b
T_9	122.4	120.4	8^a	6.5^a
CD (P = 0.05)	1.5	0.9	0.7	0.7
Shooting	**Number of shoots**			
S_1	7	8.5		
S_2	9.3	10		
S_3	11.6	12		
S_4	12.3	14.3		
CD (P = 0.05)	3.0	1.6		

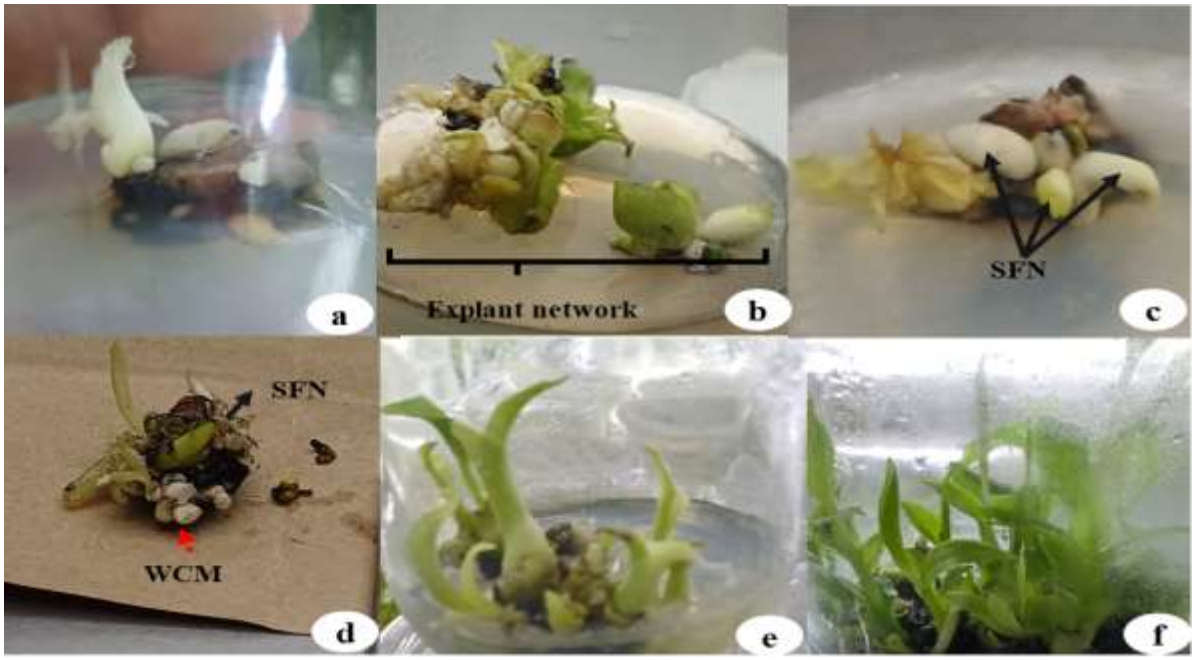

Fig. 4: *In vitro* regeneration using longitudinally sectioned small floral apex segments as explants
(a) Longitudinal section of the floral apex explants with SFNs, (b) The explant network from longitudinally sectioned floral apex segments, (c) Shoot forming Nodules in the explant network, (d) White compact meristems in the explant network, (e and f) Shoots at first and second subculture

Table 2
Effect of Cytokinin and Auxin Combinations on the initiation of SFNs and WCM from Longitudinally dissected Immature Male Bud apex Segments in Banana hybrids CO 2 and CO 3

Treatments	**Number of days taken for SFNs to be formed**		**Number of Shoot-Forming Nodules per segment**		**Number of White compact meristems**	
Initiation	**CO 2**	**CO 3**	**CO 2**	**CO 3**	**CO 2**	**CO 3**
T_1	33.6	32.4	2^e	2.2^d	4.5^d	5.1^d
T_2	28.7	30.7	3.5^c	3.5^c	6.9^c	6.9^c
T_3	27.3	29.3	4^c	5^b	7.5^b	7.5^b
T_4	31.6	31.3	3^d	2.8^d	4.8^d	5.4^d
T_5	32	29.7	3.2^d	3.5^c	5.2^d	5.2^d
T_6	27	28.4	5^b	5.2^b	6.0^c	6.4^c
T_7	25.7	26.7	5.2^b	5.5^b	7.3^b	6.7^b
T_8	23.4	23.7	5.8^a	5.5^b	8.1^a	8.5^a
T_9	21.2	21.3	6^a	6^a	9^a	9.5^a
CD (P = 0.05)	**1.5**	1.0	0.7	0.6	0.7	0.7
Shooting	**Number of shoots**					
S_1	7.5	9				
S_2	9.7	11				
S_3	12	13				
S_4	14.5	15.1				
CD (P = 0.05)	2.7	1.8				

These findings are consistent with earlier reports by[1,9,24] an increase in meristematic nodules significantly boosts the regeneration potential of Musa spp. via enhanced shoot initiation frequencies. It has been described that SFNs, when properly induced under cytokinin-rich conditions, develop into dense, organized structures (white compact meristems) that serve as precursors to shoot apices[7]. The compact meristems of 0.5 cm diameter were excised and transferred to the shooting medium; the highest shoot proliferation (Fig. 4e, f) was recorded in S_4, producing 14.5 shoots in CO 2 and 15.1 shoots in CO 3, indicating the optimal hormonal combination for maximum regeneration. This was followed by S_3, 12 shoots in CO 2 and 13 in CO 3. The lowest number of shoots, 7.5 and 9 shoots in CO 2 and CO 3, were recorded

in S_1. The observed increase in shoots with elevated levels of BAP and Kinetin reflects the synergistic role of cytokinins in promoting shoot initiation and proliferation. BAP is well known to stimulate axillary bud activation, while kinetin supports cell division and elongation.

The observations align with findings by Ali et al[1] and Madhulatha et al[15] who reported enhanced shoot multiplication rates when using a combination of cytokinins. Across all treatments, CO 3 consistently produced more shoots than CO 2, this might be due to the presence of the B genome, which has high scalp induction and proliferation potential rather than the A genome. So, bananas can be genetically manipulated for improving shoot regeneration[4]. After 21 days, the individual plantlets were separated and transferred to rooting medium supplemented with IBA @ 2 mg/l and within 21 days, the hairy roots started to develop.

Conclusion

This study demonstrates the effectiveness of using longitudinally sectioned immature male floral buds as explants for establishing multiple meristem cultures in banana hybrids CO 2 and CO 3. The optimized hormonal combinations significantly enhanced early SFN induction, meristem development and shoot proliferation in both the hybrids. The approach offers a promising alternative to traditional sucker-based propagation by enabling high-frequency, genotype-stable micropropagation.

The prospects include scaling this protocol for commercial tissue culture units, exploring its application in other banana genotypes, utilising the meristems to develop suspension cultures and integrating it with genetic transformation platforms for trait improvement and disease resistance breeding.

References

1. Ali A., Sajid A., Naveed N.H., Majid A., Saleem A., Khan U.A., Jafery F.I. and Naz S., Initiation, proliferation and development of a micro-propagation system for mass-scale production of banana through meristem culture, *Afr. J. Biotechnol.*, **10**, 15731-15738 **(2011)**

2. Arinatwe G., Rubaihayo P.R. and Magambo M.J., Proliferation rate effects of cytokinins on banana (Musa spp.) cultivars, *Sci. Hort*, **86**, 13-21 **(2000)**

3. Ashokkumar K., Elayabalan S., Shobana V.G., Sivakumar P. and Pandiyan M., Nutritional value of cultivars of Banana (Musa spp.) and its future prospects, *J. Pharmacogn. Phytochem.*, **7(3)**, 2972- 2977 **(2018)**

4. Bidabadi S.S., Meon S. and Wahab Z., Scalp induction rate responses to cytokinins on proliferating shoot-tips of banana cultivars (Musa spp.), *American Journal of Agricultural and Biological Sciences*, **5(2)**, 128-134 **(2010)**

5. Borborah K., Saikia D., Rehman M., Islam M., Mahanta S., Chutia J., Borthakur S. and Tanti B., Comparative Analysis of Genetic Diversity in Some Non-commercial Cultivars of Musa L. from Assam, India, Using Morphometric and ISSR Markers, *International Journal of Fruit Science*, **20**, 1814 – 1828 **(2020)**

6. Darvari F.M., Sariah M., Puad M.P. and Maziah M., Micropropagation of some Malaysian banana and plantain (Musa sp.) cultivars using male flowers, *African Journal of Biotechnology*, **9**, 16 **(2010)**

7. Fitramala E., Khaerunisa E., Djuita Ratna N., Sunarso H. and Ratnadewi D., *In vitro* culture of banana (Musa paradisiaca L.) cv. Kepok Merah for fast micropropagation, *Menara Perkebunan*, **84(2)**, 69-75 **(2016)**

8. Gübbük H. and Pekmezci M., *In vitro* propagation of some new banana types (Musa spp.), *Turkish Journal of Agriculture and Forestry*, **28(5)**, 355-361 **(2004)**

9. Habiba S.U., Shimasaki K., Ahasan M.M. and Alam M.M., Effect of different cytokinins on in vitro organogenesis in protocorm-like bodies (PLBs) of Epidendrum 'Rouge Star No. 8', *Middle East J. Sci. Res*, **21**, 1843-7 **(2014)**

10. Hegde D.M. and Hiwale S., Banana production in India: Past, present and future, *Indian Journal of Horticulture*, **71(1)**, 1-10 **(2014)**

11. Kalimuthu K., Saravankumar M. and Senthilkumar R., In vitro micropropagation of Musa sapientum L.(Cavendish Dwarf), *International Research Journal of Genetic Engineering*, **1(4)**, 054-056 **(2013)**

12. Kavitha N., Saraswathi M.S., Kannan G., Bathrinath M., Backiyarani S. and Uma S., Development of direct regeneration protocol for mass multiplication of Musa spp. variety Udhayam (Pisang Awak, ABB) using different explants, *Scientia Horticulturae*, **290**, 110506 **(2021)**

13. Kumar R., Singh R.K., Shah A., Srivastava A.K. and Agarwal A., Banana cultivation and micropropagation in India: Addressing challenges and exploring future prospects, *Biosciences Biotechnology Research Asia*, **21(4)**, 123-135 **(2024)**

14. Lusiyanto Nurhasanah and Sunaryo W., *In vitro* regeneration of banana genotypes possessing distinct genomes by using male flower explants, *SABRAO Journal of Breeding and Genetics*, **53(2)**, 322-333 **(2021)**

15. Madhulatha P., Anbalagan M., Jayachandran S. and Sakthivel N., Influence of liquid pulse treatment with growth regulators on in vitro propagation of banana, *Plant Cell, Tissue and Organ Culture*, **76(2)**, 189–191 **(2004)**

16. Mallick J., Das N., Roy K. and Mazumder S., An overview of various types of banana production and its market value in India, *International Research Journal of Engineering and Technology (IRJET)*, **7(12)**, 12214 **(2023)**

17. Murashige T. and Skoog F., A revised medium for rapid growth and bioassays with tobacco tissue cultures, *Physiologia Plantarum*, **15(3)**, 473-497 **(1962)**

18. National Horticulture Board, Banana production in India: Cost and yield analysis, Ministry of Agriculture & Farmers Welfare, Government of India, https://www.nhb.gov.in **(2023)**

19. Rainiyati, Lizawati and Kristiana M., Peranan IAA dan BAP terhadap perkembangan nodul pisang (Musa AAB) raja nangka secara in vitro. *J. Agron*., **13(1)**, 51-57 **(2009)**

20. Rayis S.A. and Abdallah A.A., Somatic embryogenesis for the genetic improvement of a triploid banana (Musa Spp. AAA Cv. Berangan) using three different media with different growth regulators, *Int J Recent Res Life Sci*, **2**, 53–58 **(2015)**

21. Resmi L. and Nair A.S., Plantlet production from male inflorescence tips of Musa acuminata cultivars from South India, *Plant Cell Tissue Organ Cult.*, **88**, 333–338 **(2007)**

22. Rustagi A., Jain S., Kumar D., Shekhar S., Jain M., Bhat V. and Sarin N.B., High efficiency transformation of Banana [(Musa accuminata cv. Matti (AA)] for enhanced tolerance to salt and drought stress through overexpression of a peanut Salinity-Induced Pathogenesis- Related class 10 protein, *Mol Biotecnol*, **57(1)**, 27–35 **(2015)**

23. Saraswathi M.S., Praveena S., Uma S. and Thangavelu R., Development of an efficient micropropagation technique for Musa variety Udhayam (ABB), *Indian J. Hortic*., **71**, 452–457 **(2014)**

24. Strosse H., Van den Houwe I. and Panis B., Banana cell and tissue culture: A review, Plant Cell, Tissue and Organ Culture, 1-12 **(2004)**

25. Subramaniam S., Rathinam X., Poobathy R. and Sinniah U., *In vitro* production of multiple bud clumps (Mbcs) from Cavendish banana cultivar, Brasilian (AAA), *American-Eurasian J. Sustainable Agric*, **2(3)**, 300-307 **(2008)**

26. Tripathi L. and Tripathi J.N., High frequency shoot regeneration of various cultivars of banana (Musa sp.). *Journal of Crop Improvement*, **22**, 171-180 **(2008)**

27. Wirakarnain S., Plantlet production through the development of competent multiple meristem cultures from the male inflorescence of banana, Musa acuminata cv. 'Pisang Mas' (AA), *Am. J. Biochem. Biotechnol.*, **4(4)**, 325-328 **(2008)**.

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