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# Standardization of *Meta*-Topolin Concentration for Maximizing *In vitro* Proliferation in Commercial Banana Cultivars of Andhra Pradesh, India

**R. Naga Lakshmi [a*], K. Mamatha [b], B. V. K. Bhagavan [c] and M. M. Naidu [d]**

*[a] Dr. YSRHU-Horticultural Research Station, Lam, Guntur, Andhra Pradesh, India.*
*[b] Dr. YSRHU-Horticultural Research Station, Kovvur, Andhra Pradesh, India.*
*[c] Dr. YSRHU-Horticultural Research Station, Peddapeta, Andhra Pradesh, India.*
*[d] Dr. YSRHU-Horticultural Research Station, Mahanandi, Andhra Pradesh, India.*

***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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## ABSTRACT

Cytokinins are associated with cell division, cell growth, and differentiation, are the most common plant growth regulators used in micropropagation. Aromatic cytokinin, meta-topolin (*m*T), and its derivatives have been demonstrated as alternative cytokinins in the *in vitro* multiplication of various plants. Different banana cultivars differ in *in vitro* shoot proliferation due to the difference in endogenous growth regulator content among them. A study was conducted to figure out the effect of different concentrations of meta-topolin on *in vitro* shoot proliferation of banana cultivars like Karpura chakkera keli (AAB), Mortoman (AAB), and Kovvur Bontha (ABB). *In vitro* shoot proliferation was initiated from the first subculture ($C_1$) itself at 1.5 ppm *m*T concentration in

**Corresponding author: E-mail: mnlphd@gmail.com;*

Mortoman, Kovvur Bontha, and from the second subculture ($C_2$) at 1.0 ppm *m*T concentration in Kovvur Bontha. Whereas, at all other remaining concentrations, shoot proliferation of different cultivars was initiated from the third subculture ($C_3$) only. At the end of the sixth subculture ($C_6$), the maximum number of shoots per explant (82.70) was recorded in the Kovvur Bontha cultivar at 1.5 ppm *m*T concentration. Among the cultivars, the Kovvur Bontha cultivar responded better to the meta-topolin for shoot proliferation than Karpura chakkera keli (AAB) and Mortoman (AAB).

*Keywords: Meta-topolin; banana; Kovvur Bontha; Karpura chakkera keli; Mortoman; In vitro.*

## 1. INTRODUCTION

Tissue culture is the potential technique to mass produce genetically identical plantlets that can be easily acclimatized and established in the field within a relatively short period compared to conventional propagation methods. Due to its high economic importance, huge demand for large quantities of good-quality planting material, and problems associated with conventional propagation, tissue culture was the best solution for the propagation of bananas. Banana micropropagation using shoot-tip culture has long been established [1]. Essential components in tissue culture are plant growth regulators (PGRs) such as auxins and cytokinins (CKs). A high cytokinin-to-auxin ratio is typically used to induce multiplication or regeneration [2]. The choice of cytokinin (CK) and its concentration is one of the most important factors for shoot proliferation frequency in tissue culture. Each type of cytokinin has a differential ability to induce shoot proliferation at different concentrations, which could be attributed to factors such as stability, mobility, and oxidation of cytokinins in the culture medium [3]. The cytokinin most widely used is 6-benzyladenine (BA) because of its availability, effectiveness, and affordability [4]. However, it has a few drawbacks, like morphological abnormalities observed in *Musa* spp. [5], hyperhydricity (vitrification) in Eucalyptus [6]. Other cytokinins like kinetin, 2-iP [7], and TDZ [8] were also used in the micropropagation of bananas. *Meta*-topolin is a relatively new cytokinin isolated from poplar leaves in 1975 and is closely related to BA [9,10]. In the recent past, there has been a surge of promising results with the use of aromatic cytokinin, *meta*-topolin (*m*T), and its derivatives (topolins) in tissue culture of various crops. Different banana cultivars differ in proliferation efficiency due to different levels of endogenous growth regulators. Hence, the present investigation was taken up to determine the effect of *m*T on the shoot proliferation of commercial banana cultivars grown in Andhra Pradesh.

## 2. MATERIALS AND METHODS

Healthy suckers from three popular cultivars, *viz.,* Karpura chakkera keli, Mortoman, and Kovvur Bontha, were taken for study. Karpura chakkera keli (Mysore subgroup) and Mortoman (Silk subgroup) are dessert bananas, and Kovvur Bontha (Bluggoe subgroup) is cooking banana.

The outer leaf sheaths of the sword sucker were removed by retaining five whorls of leaf primordium. The excised shoot apex was dipped in 0.1% citric acid for 10–15 minutes, followed by thorough washing under running tap water for 30 minutes. After peeling off the outer layers, explants were kept in an antibiotic solution along with Tween 20 for 30 minutes under a laminar air flow cabinet. After rigorous washing with sterile distilled water four times, the explants were kept in 75% spirit for 1-2 minutes. Washing was repeated with sterile distilled water four times. The explants were surface sterilized with 0.1% $HgC1_2$ for 18 minutes and then washed with sterile water four times. Trimmed explants from all sides were kept in an antioxidant solution, followed by thorough washing with sterile distilled water two times. The explants were once again surface-sterilised with 0.1% $HgC1_2$ for 13 minutes, followed by thorough washing with sterile distilled water four times. The explant was given a final cut and kept in an antioxidant solution, followed by thorough washing with sterile distilled water.

The explants were inoculated in modified MS media supplemented with three concentrations of *m*T (0.5 ppm, 1.0 ppm, and 1.5 ppm) in combination with 0.2 mg/l IAA along with inositol in initiation media and transferred to the same media two times at an interval of 15 days. Later, the explants were transferred to multiplication media (same as initiation media without inositol) and subcultured every 21 days up to six multiplication cycles ($C_1$ to $C_6$). At the end of each subculture, the number of shoots proliferating from each initial explant was recorded. Cultures were incubated at 26 ± 2°C

by maintaining 14 h light/10 h dark conditions under 3000 lux light intensity.

## 3. RESULTS AND DISCUSSION

### 3.1 Side Shoot Initiation

Among the different banana cultivars under study, there was a difference in the time to shoot initiation (Table 1). It was observed that side shoot proliferation was initiated in the $C_3$ subculture in banana cultivar Karpura chakkera keli at all three concentrations of *m*T. In banana cultivar Mortoman, side shoot proliferation was started in the $C_3$ subculture at 0.5 and 1.0 ppm *m*T concentrations and in the $C_1$ subculture at 1.5 ppm *m*T. However, in banana cultivar Kovvur Bontha, side shoot proliferation was initiated in the $C_1$ subculture itself at 1.5 ppm *m*T concentration, in the $C_2$ subculture at 1.0 ppm *m*T concentration, and in the $C_3$ subculture at 0.5 ppm *m*T concentration. Among three banana cultivars, early shoot initiation was observed in Kovvur Bontha at high concentrations of *m*T.

### 3.2 Side Shoot Proliferation

#### 3.2.1 Subculture wise

The response of three banana cultivars to different concentrations of exogenously applied *m*T exhibited great variation at all levels of subculture (Table 1). There was a gradual increase in the number of shoots with time in all cultivars, despite the variation in proliferation among cultivars. From the $C_1$ subculture onwards up to the third subculture ($C_3$), the number of shoots per explant was maximum (8.00) in Mortoman at 1.5 ppm *m*T concentration. From the fourth subculture ($C_4$) onwards up to the sixth subculture ($C_6$), the number of shoots per explant was maximum (13.00 and 82.70, respectively) in Kovvur Bontha at 1.5 ppm *m*T concentration. Application of a high concentration of *m*T evoked a higher degree of shoot proliferation in Kovvur Bontha than in the other two cultivars. The high cytokinin activity of *m*T was reported by several workers in banana [11,12].

#### 3.2.2 *meta*–Topolin concentration wise

The results showed that *m*T had a more positive effect on shoot regeneration. In all the cultivars under evaluation, with the increase in concentrations of *m*T, there was an enhancement in the number of shoots per explant up to the end of the sixth subculture ($C_6$) except for Karpura chakkera keli. In Karpura chakkera keli, the side shoot proliferation was highest at 1.0 ppm *m*T concentration up to the $C_5$ subculture (Fig. 1a, 1b, 1c). It can be inferred that Karpura chakkera keli is more sensitive to high (1.5 ppm) *m*T concentrations than Mortoman and Kovvur Bontha up to the $C_5$ cycle. From the results, it is evident that shoot bud proliferation rate is a function of *meta*-Topolin concentration. Similar results were also noticed in Williams banana [11], Ney Poovan banana [13], and in plantain [12] under *In vitro conditions.* Differences in sensitivity could be due to cultivar-dependent responses to the different cytokinin concentrations [1].

#### 3.2.3 Cultivar wise proliferation

Among cultivars, variation in shoot proliferation was observed. In Karpura chakkera keli (Fig. 2a), the maximum number of shoots per explant was produced at 1.0 *m*T concentration up to the $C_5$ subculture and at 1.5 *m*T concentration in the $C_6$ subculture. In Mortoman (Fig. 2b), the maximum number of shoots per explant was produced at a concentration of 1.5 *m*T in all the multiplication cycles, *i.e.,* up to the sixth subculture ($C_6$). The same trend was followed in Kovvur Bontha (Fig. 2c), where the maximum number of shoots per explant was produced at a concentration of 1.5 *m*T in all the subcultures, *i.e.,* up to the sixth subculture ($C_6$). Similarly, superior multiplication rates for *m*T treatments were reported in banana cultivars 'Williams' and 'Grand Naine' [11], in Patakpura banana [14], and in plantain [12].

At the end of the sixth subculture, the maximum number of shoots were produced per initial explant at a concentration of 1.5 *m*T in the banana cultivar Kovvur Bontha (82.70), followed by Karpura chakkera keli (18.49), and Mortoman (14.25). Variation in the degree and pattern of shoot bud proliferation is observed not only among cultivars but also within the different genomic groups. A high variation in multiplication rate is reported among species of the same genus, even when cultured under the same conditions [15]. Proliferation and multiplication *In vitro* depend, besides other factors, also on genotype [16] and are cultivar specificity [17] or cultivar dependent [18]. The varying degrees of *in vitro* shoot proliferation can also be explained by the fact that levels of inherent endogenous auxins and cytokinins differ between genotypes [19].

**Table 1. Subculture wise shoot proliferation in three banana cultivars at various concentrations of *meta-topolin***

| Subculture | Shoot proliferation in Karpura chakkera keli | | | Shoot proliferation in Mortoman | | | Shoot proliferation in Kovvur Bontha | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0.5 ppm mT | 1.0 ppm mT | 1.5 ppm mT | 0.5 ppm mT | 1.0 ppm mT | 1.5 ppm mT | 0.5 ppm mT | 1.0 ppm mT | 1.5 ppm mT |
| $C_1$ | - | - | - | - | - | 2.50 | - | - | 2.00 |
| $C_2$ | - | - | - | - | - | 5.80 | - | 2.50 | 4.10 |
| $C_3$ | 3.25 | 5.24 | 3.00 | 2.04 | 2.44 | 8.0 | 3.00 | 5.00 | 7.70 |
| C4 | 4.20 | 10.60 | 5.00 | 3.20 | 4.00 | 9.8 | 4.60 | 6.60 | 13.00 |
| C5 | 5.00 | 13.40 | 11.50 | 4.10 | 6.10 | 12.6 | 6.20 | 13.50 | 39.70 |
| C6 | 6.33 | 15.60 | 18.49 | 4.87 | 7.90 | 14.25 | 8.60 | 19.20 | 82.70 |

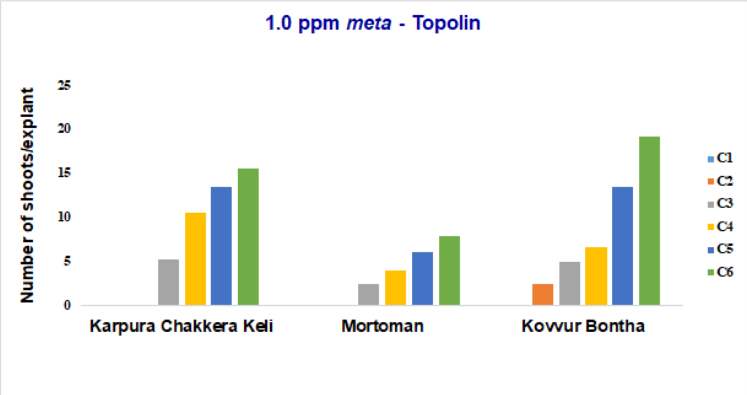


**Fig. 1b Shoot proliferation at 1.0 ppm *meta* – Topolin (*m*T) concentration**

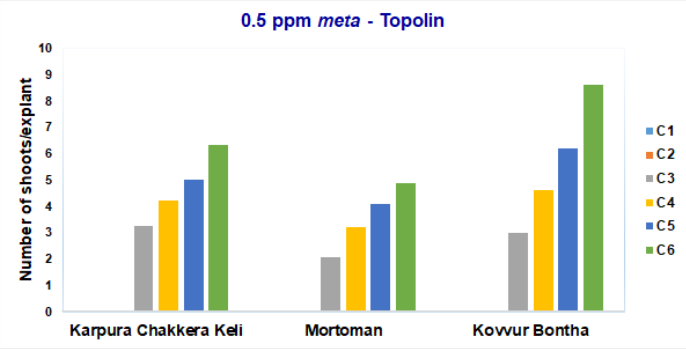


**Fig. 1a Shoot proliferation at 0.5 ppm *meta* – Topolin (*m*T) concentration**

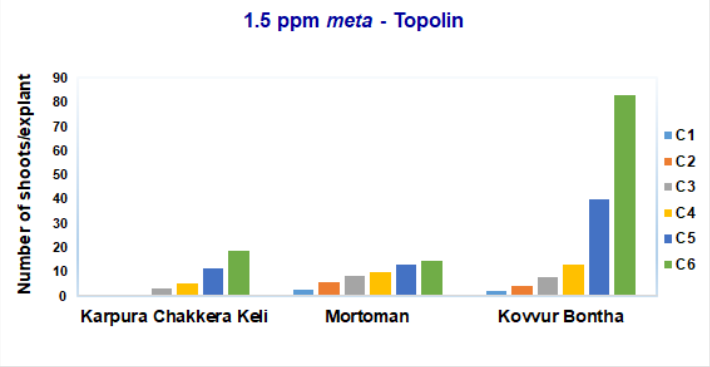


**Fig. 1c Shoot proliferation at 1.5 ppm *meta* – Topolin (*m*T) concentration**

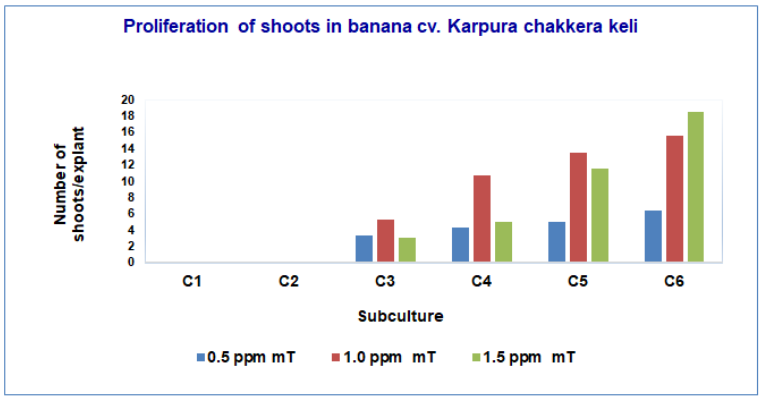


**Fig. 2a Shoot proliferation in Karpura chakkera keli at various concentrations of *meta*- topolin (*m*T)**

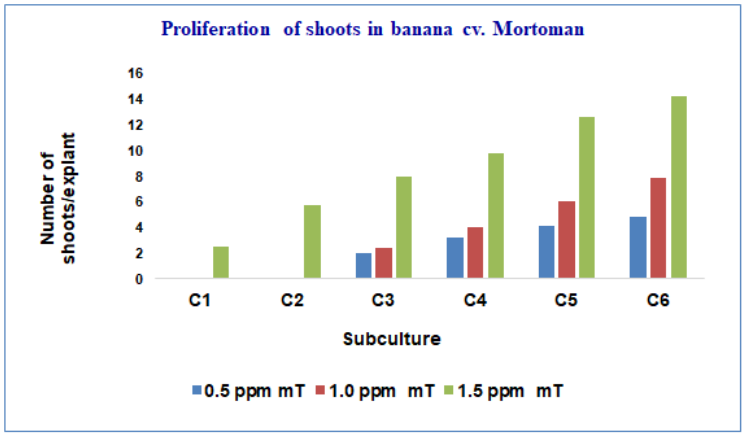


**Fig. 2b Shoot proliferation Mortoman at various concentrations of *meta*- topolin (*m*T)**

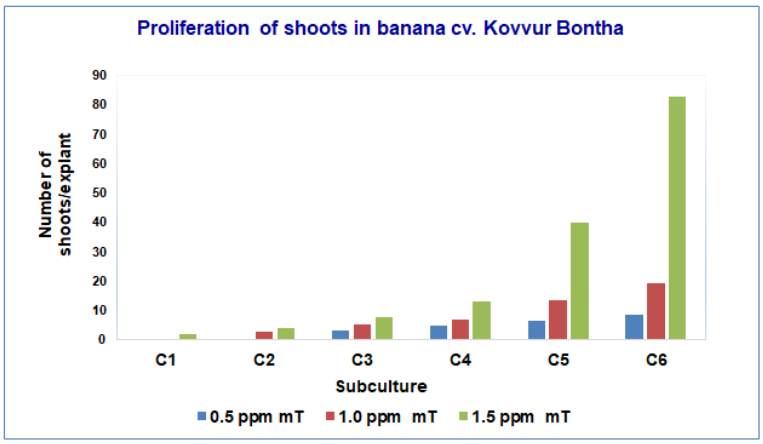


**Fig. 2c Shoot proliferation Kovvur Bontha at various concentrations of *meta*- topolin (*m*T)**

Bananas showed a wide range of dose-dependent responses among and within the genomic groups of the *Eu Musa* series [11]. The presence of 'B' in the genotype adversely affected multiplication; the more 'B' genomes in the group, the lower the rate of multiplication [17]. However, from the investigation, it is inferred that Kovvur Bontha (ABB) responded well to *m*T and produced the highest number of shoots per explant, even though it has two B genomes. This might be due to the fact that the effect of the B genome was abolished by *m*T and produced more shoots in Kovvur Bontha than the other two cultivars having one B genome.

## 4. CONCLUSIONS

In three 'B' genome containing bananas, *viz.,* Karpura chakkera keli (AAB), Mortoman (AAB), and Kovvur Bontha (ABB), shoot proliferation was influenced by *meta*-topolin. At the end of the sixth subculture ($C_6$), the maximum number of shoots was produced per initial explant at a concentration of 1.5 *m*T in the banana cultivar Kovvur Bontha (ABB), which is better than Karpura chakkera keli (AAB) and Mortoman (AAB). Hence, *meta*-topolin could be used for more shoot proliferation in Kovvur Bontha.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES


1. Vuylsteke DR. Shoot-tip culture for the propagation, conservation, and distribution of *Musa* Germplasm. International Institute of Tropical Agriculture: Ibadan, Nigeria; 1998.
2. Murashige T, Skoog T. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum.1962;15:473–97.
3. Buah JN, Danso E, Taah KJ, Abole EA, Bediako EA, Asiedu J, Baidoo R. The effects of different concentrations of cytokinins on the *In vitro* multiplication of plantain (*Musa* sp.). Biotechnology. 2010;9:343-47.
4. Bairu MW, Stirk WA, Dolezˇal K, Van Staden J. Optimizing the micropropagation protocol for the endangered Aloe polyphylla: can meta-topolin and its derivatives serve as replacement for benzyladenine and zeatin? Plant Cell, Tissue and Organ Culture. 2007;90: 15-23.
5. Shirani S, Mahdavi F, Maziah M. Morphological abnormality among regenerated shoots of banana and plantain (*Musa* spp.) after *in vitro* multiplication with TDZ and BAP from excised shoot tips. African Journal of Biotechnology. 2009;8(21):5755-61.
6. Van der Westhuizen A. The use of meta-topolin as an alternative cytokinin in the tissue culture of Eucalyptus species. Acta Horticulturae. 2014;1055:25-28.
7. Resmi L, Nair AS. Differential effect of cytokinins in the micropropagation of diploid and triploid Musa cultivars. International Journal of Integrative Biology. 2011;11(1):35-38.
8. Manjula R, Praveen J, Sunnaiah KV, Swamy GSK, Prabhuling G. Enhancement of *In vitro* shoot multiplication in banana cv. Rajapuri (AAB) using TDZ. Research Journal of Biotechnology. 2015;10(5):5-10.
9. Strnad M, Hanuš J, Vaněk T, Kaminek M, Ballantine JA, Fussel B, Hanke DE. *Meta*-topolin, a highly active aromatic cytokinin from poplar leaves (*Populus* × *canadensis* Moench., cv. Robusta). Phytochemistry. 1997;45:213-18.
10. Teklehaymanot T, Wannakrairoj S, Pipattanawong N. *Meta*-topolin for pineapple shoot multiplication under three in vitro systems. American-Eurasian Journal of Agricultural and Environmental Sciences. 2010;7:157-62.
11. Bairu MW, Stirk WA, Dolezˇal K, Van Staden J. The role of topolins in micropropagation and somaclonal variation of banana cultivars 'Williams' and 'Grand Naine' (*Musa* spp. AAA). Plant Cell, Tissue Organ Culture. 2008; 95:373-79.
12. Escalona M, Cejas I, Olmedo JG, Capote I, Roels S, Cañal MJ, Rodriguez R, Sandoval JO, Debergh P. The effect of *meta*-topolin on plantain propagation using a temporary immersion bioreactor. Infomusa. 2003;12:28-30.
13. Bohra P, Waman AA, Sathyanarayana BN, Umesha K, Gowda B. Influence of different growth regulators on *In vitro* multiplication of mixed diploid banana (*Musa* AB). Proceedings of the National Academy of Sciences, India Section B: Biological Sciences. 2014;86:179-85.
14. Mohapatra N, Deo B. Substitution of BAP with meta-Topolin (m-T) in multiplication culture of Musa species. Plant Science Research. 2019;41(1&2):8-11.
15. George EF, Sherrington PD. Plant propagation by tissue culture. Handbook and directory of commercial laboratories. Exegetics Ltd: UK; 1988.
16. Israeli Y, Lahav E, Reuveni O. *In vitro* culture of bananas. In: Gowen S. editor. Bananas and Plantains. Chapman and Hall: London; 1995.
17. Hirimburegama K, Gamage N. Cultivar specificity with respect to *In vitro*

micropropagation of *Musa* spp. (banana and plantain). Journal of Horticultural Sciences. 1997;72:205-11.

18. Arinaitwe G, Rubaihayo PR, Magambo MJS. Proliferation rate effects of cytokinins on banana (*Musa* spp.) cultivars. Scientia Horticulturae. 2000;86:13-21.
19. Pierik RLM. *In vitro* Culture of Higher Plants. Martinus Nijhoff: Boston; 1987.



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