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

Original research

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 (*mT*), and its derivatives have been demonstrated as alternative cytokinins in their *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 chakker 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 *mT* concentration in Mortoman, Kovvur Bontha, and from the second subculture (C_2) at 1.0 ppm *mT* 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 *mT* concentration. Among the cultivars, the Kovvur Bontha cultivar responded better to the meta-topolin for shoot proliferation than Karpura chakkerakeli (AAB) and Mortoman (AAB).

Keywords: meta-topolin, banana, Kovvur Bontha, Karpura chakkerakeli, 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 [19]. 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 [12]. 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 [5]. The cytokinin most widely used is 6-benzyladenine (BA) because of its availability, effectiveness, and affordability [2]. However, it has a few drawbacks, like morphological abnormalities observed in *Musa* spp. [15], hyperhydricity (vitrification) in *Eucalyptus* [18]. Other cytokinins like kinetin, 2-iP [14], and TDZ [10] 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 [16], [17]. In the recent past, there has been a surge of promising results with the use of aromatic cytokinin, *meta*-topolin (*mT*), 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 *mT* 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% HgCl₂ 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% HgCl₂ 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 *mT* **(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 (C₁ to C₆). 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

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** C₃ subculture in banana cultivar Karpura chakkera keli at all three concentrations of *mT*. In banana cultivar Mortoman, side shoot proliferation was started in **the** C₃ subculture at 0.5 and 1.0 ppm *mT* concentrations and in **the** C₁ subculture at 1.5 ppm *mT*. **However,** in banana cultivar Kovvur Bontha, side shoot proliferation was initiated in **the** C₁ subculture **itself** at 1.5 ppm *mT* concentration, in **the** C₂ subculture at 1.0 ppm *mT* concentration, and in **the** C₃ subculture at 0.5 ppm *mT* concentration. **Among three banana cultivars, early shoot initiation was observed in Kovvur Bontha at high concentrations of *mT*.**

Table1 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
C ₄	4.20	10.60	5.00	3.20	4.00	9.8	4.60	6.60	13.00
C ₅	5.00	13.40	11.50	4.10	6.10	12.6	6.20	13.50	39.70
C ₆	6.33	15.60	18.49	4.87	7.90	14.25	8.60	19.20	82.70

Side shoot proliferation

Subculture wise

The response of three banana cultivars to different concentrations of exogenously applied mT 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 C₁ subculture onwards up to the third subculture (C₃), the number of shoots per explant was maximum (8.00) in Mortoman at 1.5 ppm mT concentration. From the fourth subculture (C₄) onwards up to the sixth subculture (C₆), the number of shoots per explant was maximum (13.00 and 82.70, respectively) in Kovvur Bontha at 1.5 ppm mT concentration. Application of a high concentration of mT evoked a higher degree of shoot proliferation in Kovvur Bontha than in the other two cultivars. The high cytokinin activity of mT was reported by several workers in banana [3], [6].

meta-Topolin concentration wise

The results showed that mT had a more positive effect on shoot regeneration. In all the cultivars under evaluation, with the increase in concentrations of mT, there was an enhancement in the number of shoots per explant up to the end of the sixth subculture (C₆) except for Karpura chakkera keli. In Karpura chakkera keli, the side shoot proliferation was highest at 1.0 ppm mT concentration up to the C₅ subculture (Fig. 1a,

1b, 1c). It can be inferred that Karpura chakkera keli is more sensitive to high (1.5 ppm) *mT* concentrations than Mortoman and Kovvur Bontha up to the C₅ 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 [3], Ney Poovan banana [4], and in plantain [6] under *in vitro* conditions. Differences in sensitivity could be due to cultivar-dependent responses to the different cytokinin concentrations [19].

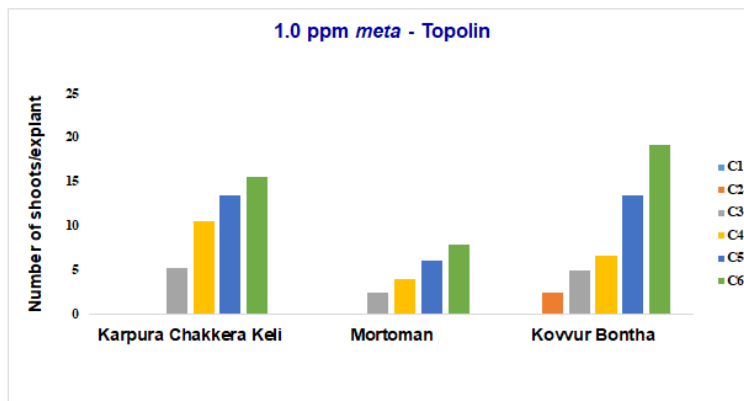


Fig. 1b Shoot proliferation at 1.0 ppm *meta* – Topolin (*mT*) concentration

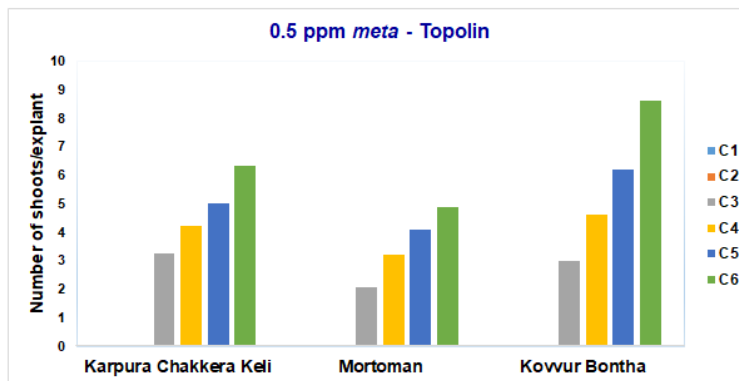


Fig. 1a Shoot proliferation at 0.5 ppm *meta* – Topolin (*mT*) concentration

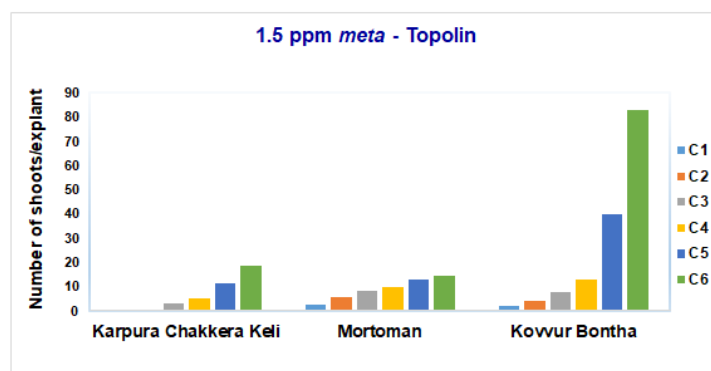


Fig. 1c Shoot proliferation at 1.5 ppm meta – Topolin (mT) concentration

Cultivar wise proliferation

Among cultivars, variation in shoot proliferation was observed. In Karpura chakkerakeli (Fig. 2a), the maximum number of shoots per explant was produced at 1.0 mT concentration up to the C₅ subculture and at 1.5 mT concentration in the C₆ subculture. In Mortoman (Fig.2b), the maximum number of shoots per explant was produced at a concentration of 1.5 mT in all the multiplication cycles, i.e., up to the sixth subculture (C₆). The same trend was followed in Kowvur Bontha (Fig.2c), where the maximum number of shoots per explant was produced at a concentration of 1.5 mT in all the subcultures, i.e., up to the sixth subculture (C₆). Similarly, superior multiplication rates for mT treatments were reported in banana cultivars 'Williams' and 'Grand Naine' [3], in Patakpura banana [11], and in plantain [6].

At the end of the sixth subculture, the maximum number of shoots were produced per initial explant at a concentration of 1.5 mT in the banana cultivar Kowvur Bontha (82.70), followed by Karpura chakkerakeli (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 [7]. Proliferation and multiplication *in vitro* depend, besides other factors, also on genotype [9] and are cultivar specificity [8] or cultivar dependent [1]. 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 [13].

Bananas showed a wide range of dose-dependent responses among and within the genomic groups of the *Eu Musa* series [3]. The presence of 'B' in the genotype adversely affected multiplication; the more 'B' genomes in the group, the lower the rate of multiplication [8]. However, from the investigation, it is inferred that Kovvur Bontha (ABB) responded well to *mT* 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 *mT* and produced more shoots in Kovvur Bontha than the other two cultivars having one B genome.

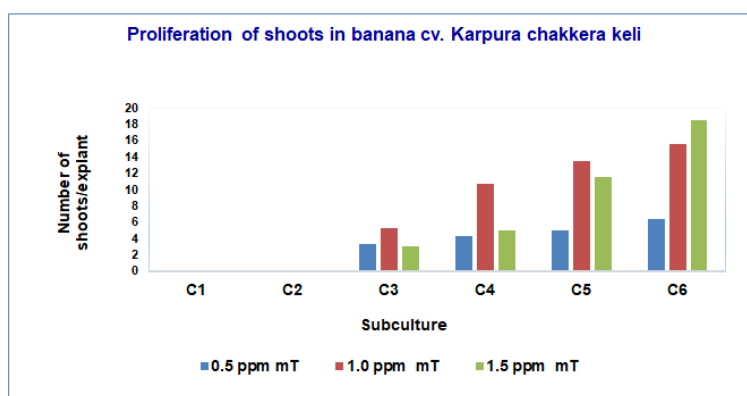


Fig. 2a Shoot proliferation in Karpura chakkera keli at various concentrations of *meta*-topolin (*mT*)

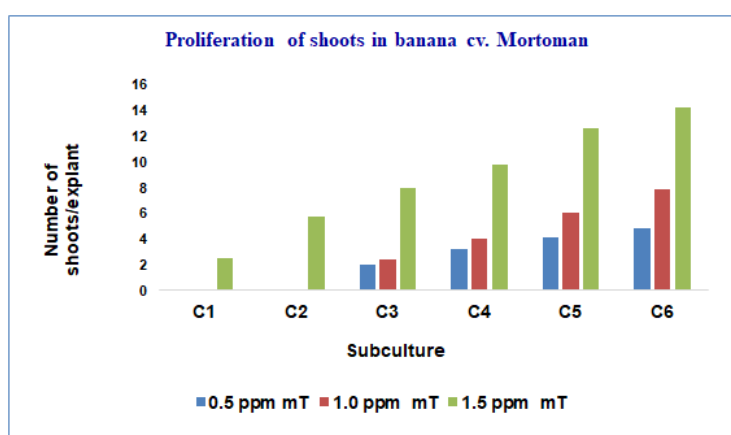


Fig. 2b Shoot proliferation Mortoman at various concentrations of *meta*-topolin (*mT*)

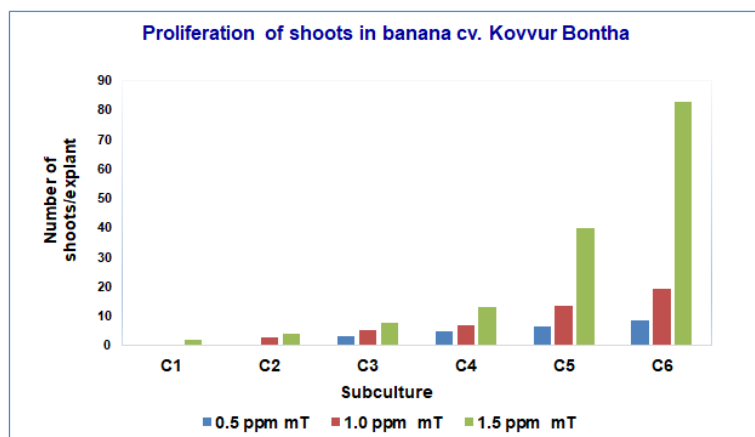


Fig. 2c Shoot proliferation Kovvur Bontha at various concentrations of meta-topolin (mT)

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 (C₆), the maximum number of shoots was produced per initial explant at a concentration of 1.5 mT in the banana cultivar Kovvur Bontha (ABB), which is better than Karpura chakkerakeli (AAB) and Mortoman (AAB). Hence, meta-topolin could be used for more shoot proliferation in Kovvur Bontha.

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