Original Research Article

Standardization of *Meta*-Topolin Concentration for Maximizing *in vitro* Proliferation in banana cultivars

Abstract

Cytokinins are associated with cell division, cell growth, differentiation and are most common plant growth regulators used in micro propagation. Aromatic cytokinin, meta-topolin (mT) and its derivatives have been demonstrated as alternative cytokinins in in vitro multiplication of various plants. Different banana cultivars differ in in vitro shoot proliferation due to the difference in endogenous growth regulators content among them. A study was conducted to know the effect of different concentrations of meta-topolin on in vitro shoot proliferation of banana cultivars like Karpurachakkerakeli (AAB), Mortoman (AAB), KovvurBontha (ABB). In vitro shoot proliferation was initiated from first subculture (C1) itself at 1.5 ppm mT concentration in Mortoman, KovvurBontha and from second subculture (C2) at 1.0 ppm mT concentration in KovvurBontha. Whereas, at all other remaining concentrations, shoot proliferation of different cultivars was initiated from third subculture (C3) only. At the end of sixth subculture (C6), maximum number of shoots per explant (82.70) was recorded in KovvurBontha cultivar at 1.5 ppm mT concentration. Among the cultivars, KovvurBontha cultivar responded well to the meta-topolin for shoot proliferation than Karpurachakkerakeli (AAB), Mortoman (AAB).

Keywords:meta-topolin, banana, KovvurBontha, Karpurachakkerakeli, Mortoman, in vitro

1 Introduction

Tissue culture is the potential technique to mass produce genetically identical plantlets which can be easily acclimatized and established in the field within a relatively short period compared to the conventional propagation methods. Due to high economic importance, huge demand for large quantities of good quality planting material and problems associated with conventional propagation made tissue culture as the best solution in the propagation of banana. Banana micro propagation using

shoot-tip culture has long been established [12]. Essential components in tissue culture are plant growth regulators (PGRs) such as auxins and cytokinins (CKs). A high cytokinin auxin ratio is typically used to induce multiplication or regeneration [6]. 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 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 [1]. However, it has a few drawbacks like morphological abnormalities observed in *Musa* spp. [8], hyperhydricity (vitrification) in Eucalyptus reported by Van der Westhuizen [11]. Other cytokinins like kinetin and 2-iP were also used in the micro propagation of banana [5] *and TDZ* [7].*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 study was taken up to know the effect of *m*T on the shoot proliferation of commercial banana cultivars grown in Andhra Pradesh.

2 MaterialsandMethods

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

Outer leaf sheaths of 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 of outer layer, explants were kept in antibiotic solution along with Tween 20 for 30 minutes under Laminar Air Flow Cabinet. After washing with sterile distilled water for four times the explants were kept in 75% spirit for 1-2 minutes. Again washed with sterile distilled water for four times and the explants were surface sterilized with 0.1% $HgC1_2$ for 18

minutes and then washed with sterile water for four times. Trimmed explants from all sides were kept in antioxidant solution followed by thorough washing with sterile distilled water for two times. Explants were again surface sterilised with 0.1% $HgC1_2$ for 13 minutes followed by thorough washing with sterile distilled water for four times. The explant was given a final cut and kept in antioxidant solution and washed thoroughly with sterile distilled water.

The explants were inoculated in modified MS media supplemented with three concentrations of *m*T in combination with 0.2 mg/l 1AA 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 sub cultured for every 21 days up to six multiplication cycles (C_1 to C_6). At end of each sub culture recorded the number of shoots proliferated from each initial explant. Cultures were incubated in a growth room having 14 h light/10 h dark conditions with light intensity of 3000 lux at 26 ± 2^0C.

3 ResultsandDiscussion

Side shoot initiation

From the Table 1, it was observed that side shoot proliferation was initiated in C_3 subculture in banana cv. Karpurachakkerakeli at all the three concentrations of *m*T. In banana cv. Mortoman side shoot proliferation was started in C_3 subculture at 0.5 and 1.0 ppm *m*T concentration and in C_1 subculture at 1.5 ppm *m*T. Whereas in banana cv. KovvurBontha side shoot proliferation was initiated in C_1 subculture at 1.5 ppm *m*T concentration, in C_2 at 1.0 ppm *m*T and in C_3 at 0.5 ppm *m*T.

Table1 Subculture wise shoot proliferation in three banana cultivars at various concentrations of *meta*- topolin

Subculture	Shoot proliferation in Karpurachakkerakeli			Shoot proliferation in Mortoman			Shoot proliferation in KovvurBontha		
	0.5 ppm *m*T	1.0 ppm *m*T	1.5 ppm *m*T	0.5 ppm *m*T	1.0 ppm *m*T	1.5 ppm *m*T	0.5 ppm *m*T	1.0 ppm *m*T	1.5 ppm *m*T
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

Sub culture wise

- Response of different genotypes to different concentrations of exogenously applied cytokinins exhibit great variations (Table 1). From C_1 multiplication cycle onwards up to third multiplication cycle (C_3) the number of shoots per explant was maximum (8.00) in Mortoman at 1.5 ppm *m*T concentration. From fourth multiplication cycle (C_4) onwards up to sixth cycle (C_6) the number of shoots per explant was maximum (13.00 and 82.70 respectively) in KovvurBontha at 1.5 ppm *m*T concentration.

meta – Topolin concentration wise

- With the increase in the concentrations of *m*T in all the cultivars under study there was an increase in the number of shoots per explant up to the end of sixth subculture (C_6) except for Karpurachakkerakeli where the side shoot proliferation was maximum at 1.0 ppm *m*T concentration up to C_5 cycle (Fig. 1a,1b,1c). It can be inferred that Karpurachakkerakeli is more sensitive to high (1.5 ppm) *m*T concentration than Mortoman and KovvurBontha up to C_5 cycle. From the results, it is evident that shoot bud proliferation rate is a function of cytokinin (*meta* – Topolin) concentration.

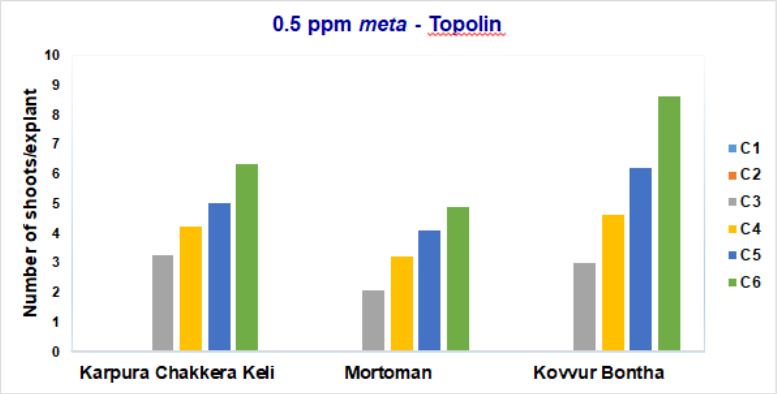

Fig. 1a Shoot proliferation at 0.5 ppm *meta* – Topolin (*m*T) concentration

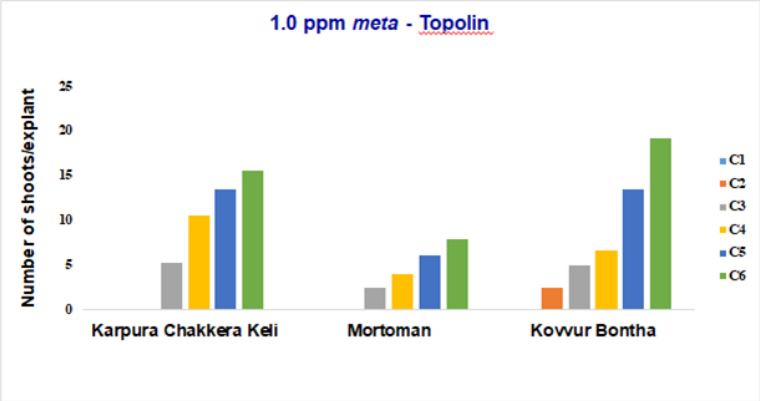

Fig. 1b Shoot proliferation at 1.0 ppm *meta* – Topolin (*m*T) concentration

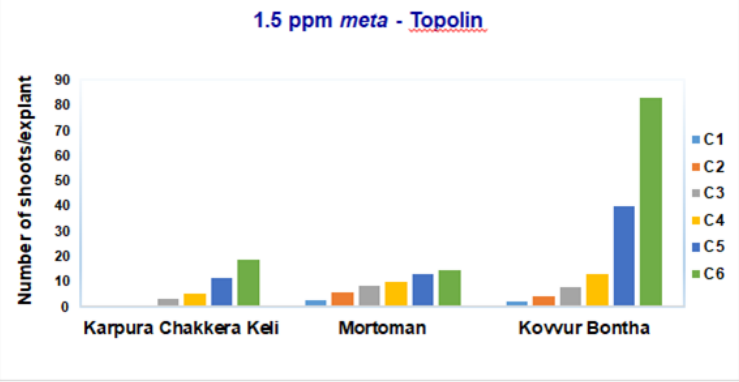

Fig. 1c Shoot proliferation at 1.5 ppm *meta* – Topolin (*m*T) concentration

Cultivar wise proliferation

- In Karpurachakkerakeli (Fig. 2a), maximum number of shoots per explant was produced at 1.0 *m*Tconcentration up to C_5 cycle and at 1.5 *m*T concentration in C_6 subculture. In Mortoman (Fig.2b), maximum number of shoots per explants was produced at 1.5 *m*Tconcentrationin all the multiplication cycles *i.e.,* up to sixth subculture (C_6). Same trend was followed in KovvurBontha (Fig.2c), maximum number of shoots per explant was produced at 1.5 *m*T concentration in all the subcultures *i.e.,* up to sixth subculture (C_6).
- At the end of sixth subculture maximum number of shoots were produced per initial explant at 1.5 *m*T concentration in banana cv. KovvurBontha (82.70) followed by Karpurachakkerakeli (18.49) and Mortoman (14.25). The presence of a B genome in a cultivar's genomic makeup could play a role in the achievement of high proliferation rate [13]. Variation in the degree and pattern of shoot bud proliferation is observed not only among cultivars, but also within the different genomic groups. Presumably, variation in the multiplication rate is due to different cultivar-dependent responses to cytokinin concentration in the medium. The varying degrees of *in vitro* shoot proliferation suggest that levels of endogenous growth regulator differ between genotypes.
- Bananas show a wide range of dose dependent responses among and within the genomic groups of *Eu Musa* series [2]. Differences in sensitivity could be due to cultivar dependent responses to the different cytokinin concentrations [13]. These results are in conformity with the findings of [4] who reported that *m*T improved multiplication of plantain. Similarly, superior multiplication rates for *m*T treatments in banana cultivars (AAA) 'Williams' and 'Grand Naine' were reported[2].

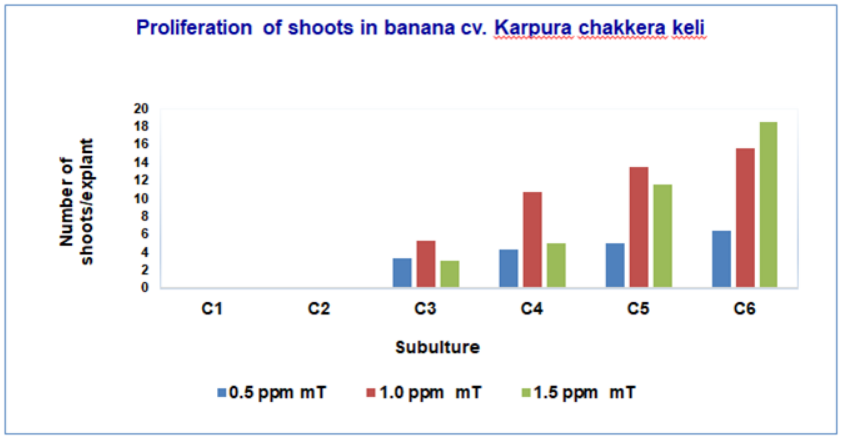

Fig. 2a Shoot proliferation in Karpura chakkera keli at various concentrations of *meta*- topolin

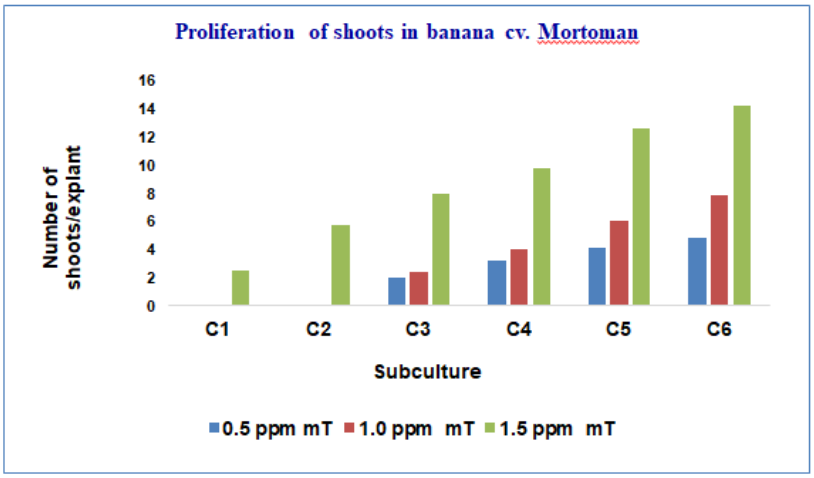

Fig. 2b Shoot proliferation Mortoman at various concentrations of *meta*- topolin

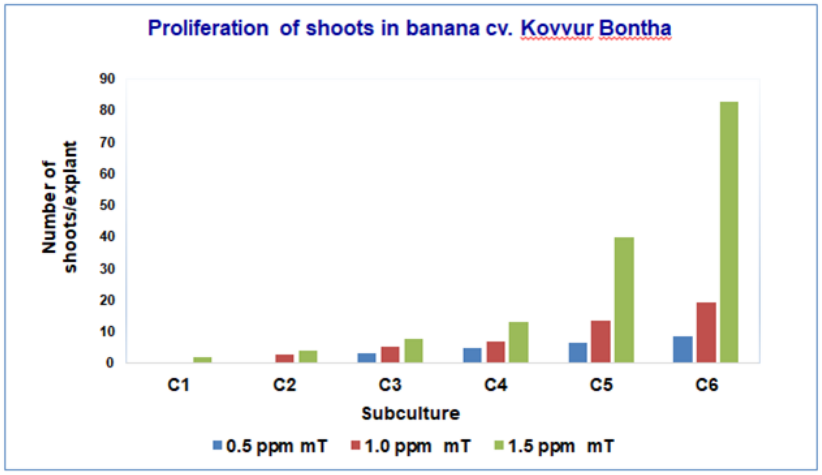

Fig. 2c Shoot proliferation Kovvur Bontha at various concentrations of *meta*- topolin

4 CONCLUSIONS

Shoot proliferation in three 'B' genome containing banana *viz.,* Karpurachakkerakeli (AAB), Mortoman (AAB) and KovvurBontha (ABB) was influenced by *meta*-topolin. At the end of sixth subculture (C_6) maximum number of shoots was produced per initial explant at 1.5 *m*Tconcentration in banana cv. KovvurBontha (ABB) compared to Karpurachakkerakeli (AAB) and Mortoman (AAB). Hence, *meta*-topolincould be used for more shoot proliferation in KovvurBontha.

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