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Influence of different cytokinins on *in vitro* shoot multiplication of banana, cv. Kovvur Bontha (ABB)

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Abstract

Bananas and plantains (*Musa* spp.) are the most critical socio-economic crops globally, being a staple food for millions of people in the tropics and also an essential commodities for the export market, including the sub-tropics. Vegetative propagation through sword suckers is slow and may carry harmful pathogens from one field to another, causing huge losses to farmers. Although frequently used in banana crops, Cavendish genotypes account for the majority of micro-propagation. Due to the absence of effective *in vitro* multiplication procedures, the production of tissue culture plant material is severely restricted in culinary genotypes. This experiment was undertaken to standardize the suitable growth regulator and its concentration on *in vitro* shoot proliferation of banana, cv. Kovvur Bontha (ABB) at Dr. YSRHU-Horticultural Research Station, Kovvur, Andhra Pradesh, during 2022-23 in a Completely Randomized Design (CRD). Banana cv. Kovvur Bontha (ABB) apical meristems were used as explants. Among the different plant growth hormones evaluated, the maximum number of shoots per culture (33.42) and maximum rate of shoot multiplication (3.11) resulted when the apical meristems were cultured in MS medium fortified with 4.0 mg/l BAP and 0.2 mg/l IAA up to the fifth subculture. Early shoot initiation (5.14 days) was noticed when the explants cultured on 3.0 mg/l BAP + 0.2 mg/l IAA up to fifth subculture. This media will facilitate the rapid multiplication of tissue culture plants in culinary banana genotypes.

Keywords: *Musa*, bluggoe, culinary, plantain, micro-propagation, shoot proliferation, growth regulators

Introduction

A valuable source of nutrition and energy, as well as a valuable means of generating cash through domestic and international trade, the banana (*Musa* spp.) is a fruit crop that is economically significant in many developing nations. It is commonly farmed in the tropics and subtropics in a wide range of agricultural systems, from modest, mixed subsistence gardens to enormous commercial monocultures. In many countries, the crop serves as a staple food or the cornerstone of the country's economy. In Andhra Pradesh, cultivated area of banana of 1 lakh Ha. Among the different genotypes of banana, Kovvur bontha (ABB, Bluggoe) is the foremost cooking variety of Andhra Pradesh and adjoining States. The fruits are long, slightly curved, with a blunt apex and prominent ridges. The rind is thick and green, with a whitish pulp. The male bud is also used for culinary purposes. This genotype is occupied in an area of 3000 ha in Andhra Pradesh. This genotype is highly susceptible to bacterial wilt caused by *Erwinia carotovora* ssp. *Carotovora* by using infected suckers as propagation material, farmers are unknowingly transferring this deadly disease from one farm to another. This problem can also be mitigated by planting disease-free tissue culture plants as planting material. Further, tissue culture plants are more productive, uniform in nature, and pest and disease free. Effective *in vitro* propagation techniques and also fine-tuning of the current procedures are being tested in various laboratories for quick multiplication and also getting disease-free and healthy plants. Additionally, a deeper comprehension of the characteristics connected to the various stages of multiplication and transfer of the land is enormously crucial for designing a successful protocol for the propagation of diverse cultivars of banana.

For the *in vitro* regeneration of different plants, growth regulators including auxin, cytokinin, gibberellin, and abscisic acid were used (Ali *et al.*, 2014; Momena *et al.*, 2014) [3, 15]. These include kinetin, indole-3-acetic acid, naphthalene acetic acid, meta-topolin, Benzylaminopurine, etc. Cytokinins like Benzylaminopurine (BAP) have been shown to increase axillary and adventitious shoot development from meristematic explants in bananas, as well as to decrease apical meristem dominance (Madhulatha *et al.*, 2004) [13].

The purpose of this research was to figure out the ideal dose of multiple growth regulators for plants for banana cv. Kovvur Bontha through *in vitro* shoot proliferation in light of the limitations associated with the production of disease-free material for planting in banana cv. Growth regulators, namely auxin, cytokinin, gibberellin, and abscisic acid like kinetin, indole-3-acetic acid, naphthalene acetic acid, thidiazuron, meta-topolin, benzylaminopurine, *etc.*, were used for the *in vitro* regeneration of various plants (Ali *et al.*, 2014; Momena *et al.*, 2014) [3, 15].

Materials and Methods

The experiment was conducted at Dr. YSRHU- Horticultural Research Station, Kovvur, during the years 2022–2023. The experiment was conducted in a Completely Randomized Design (CRD). Banana cv. Kovvur Bontha (ABB) apical meristems were used as explants. The apical meristems were obtained from well-developed suckers (About Four Months Age) of banana cv. Kovvur Bontha grown under field conditions after mother plant selection. The outer tissues and roots of the suckers were removed with the help of a sharp knife. The suckers were washed thoroughly under running tap water. The explants were cultured in sterilized media after following the standard pre-treatment and surface sterilization methods by treating the explants with bavistin, mancozeb, plantomycin, 4.0% NaOCl, 0.1% HgCl₂, 80% ethanol, *etc.* Each culture bottle contained 50 ml of MS medium with varying amounts of growth regulators, a cap, and a wrapper. The plantlets were promptly inoculated in the culture bottles. The culture bottles were moved to the growth room where they were given a regulated environment to grow in. An air conditioner kept the growth room's temperature within 25±1 °C. For the culture to grow and flourish, a 16-hour period of light was kept with a 3000 lux light intensity.

Statistical Analysis

According to the methodology used in the corresponding experiments, OPSTAT was used to statistically analyze the data gathered for each parameter involved in this investigation. Following the guidelines provided by Panse and Sukhatme (1985) [17], an analysis of variance (ANOVA) was conducted. The F table value was used to test for statistical significance at a level of 5%. Every time differences between treatment means were found to be significant, the crucial difference value at the 5% level of significance was determined. Each time, the necessary standard errors of the mean (SE m) were computed.

Results and Discussion

The research was aimed at studying the influence of different plant growth regulators, on *in vitro* shoot multiplication in banana cv. Kovvur Bontha (ABB). The experiment was conducted in the following stages: Multiplication Phase (M₁), first subculture (C₁), second subculture (C₂), third subculture (C₃), fourth subculture (C₄), and fifth subculture (C₅).

Number of shoots per culture

The effect of various hormone types and their concentrations on the average shoot number in banana cv. Kovvur Bontha had represented in table 1 and fig 1. Number of shoots indicates the influence of various plant growth hormones on the explants for the better growth and maximization of the plantlet production through tissue culture.

Multiplication phase (M₁)

As per the data envisaged in Table 1, statistically there was no statistically significant difference among the treatments for number of shoots per culture.

First subculture (C₁)

All the treatments had shown statistically significant differences for the number of shoots per culture as per the data presented in Table 1. The media supplemented with mT 6.0 mg/l (T₈) had the maximum number of shoots per explant (7.8) preceded by BAP 4.0 mg/l + IAA 0.2 mg/l (T₃) (7.3 shoots) and statistically superior with all other treatments. The explants cultured on the media subsidized with TDZ 1.0 mg/l (T₆) showed the least number of shoots per culture (1.1 shoots).

Second subculture (C₂)

Statistically significant difference was observed for the number of shoots per explant as per the data presented in the Table 1 with increase in the number of shoots per explant compared to previous subculture (C₁). The second subculture recorded the 11.28 shoots per explants, when the meristems cultured on the MS media fortified with BAP 4.0 mg/l + IAA 0.2 mg/l (T₃) which was statistically on par with mT 4.0 mg/l (T₈) (10.2 shoots) and superior with all other treatments. The minimum number of shoots (1.08) were obtained when the explants cultured on the TDZ 0.5 mg/l (T₅) and considered as the least treatment.

Third subculture (C₃)

According to the Table 1, significant difference was observed among the different treatments. The maximum number of shoots (15.3) per culture were obtained when the explants were cultured on the MS media supplemented with BAP 4.0 mg/l + IAA 0.2 mg/l (T₃) which was statistically on par with mT 6.0 mg/l (T₈) (15.1 shoots) and was followed by the explants subsidized with BAP 4.0 mg/l + Kinetin 1.0 mg/l + IAA 0.2 mg/l (T₉) (14.3 shoots). The explants cultured on media TDZ 0.5 mg/l (T₅) had shown the least number of shoots per culture (1.8) resulting in the least good treatment for culture (table 1). The performance of the cultures was shown in Fig. 1.

Fourth subculture (C₄)

Significant difference was observed across the treatment for the number of shoots per explants in fourth subculture. When explants were cultured on medium supplemented with BAP 4.0 mg/l + IAA 0.2 mg/l (T₃) the maximum number of shoots (28.2) per culture was achieved, which was statistically superior to all other treatments followed by the explants cultured on the media fortified with mT 6.0 mg/l (T₈) had shown 25.5 shoots. The least number of shoots were recorded when the explants were cultured on the media fortified with TDZ 0.5 mg/l (T₅) (1.8 shoots), making it undesirable for *in vitro* culture (Table 1).

Fifth subculture (C₅)

The representation of the data in the table 1 was evident for the presence of significant difference among treatments. Explants cultured on the media supplemented with BAP 4.0 mg/l + IAA 0.2 mg/l (T₃) resulted highest number of shoots per culture (33.4) which was statistically superior over all the other treatments followed by the explants cultured on the

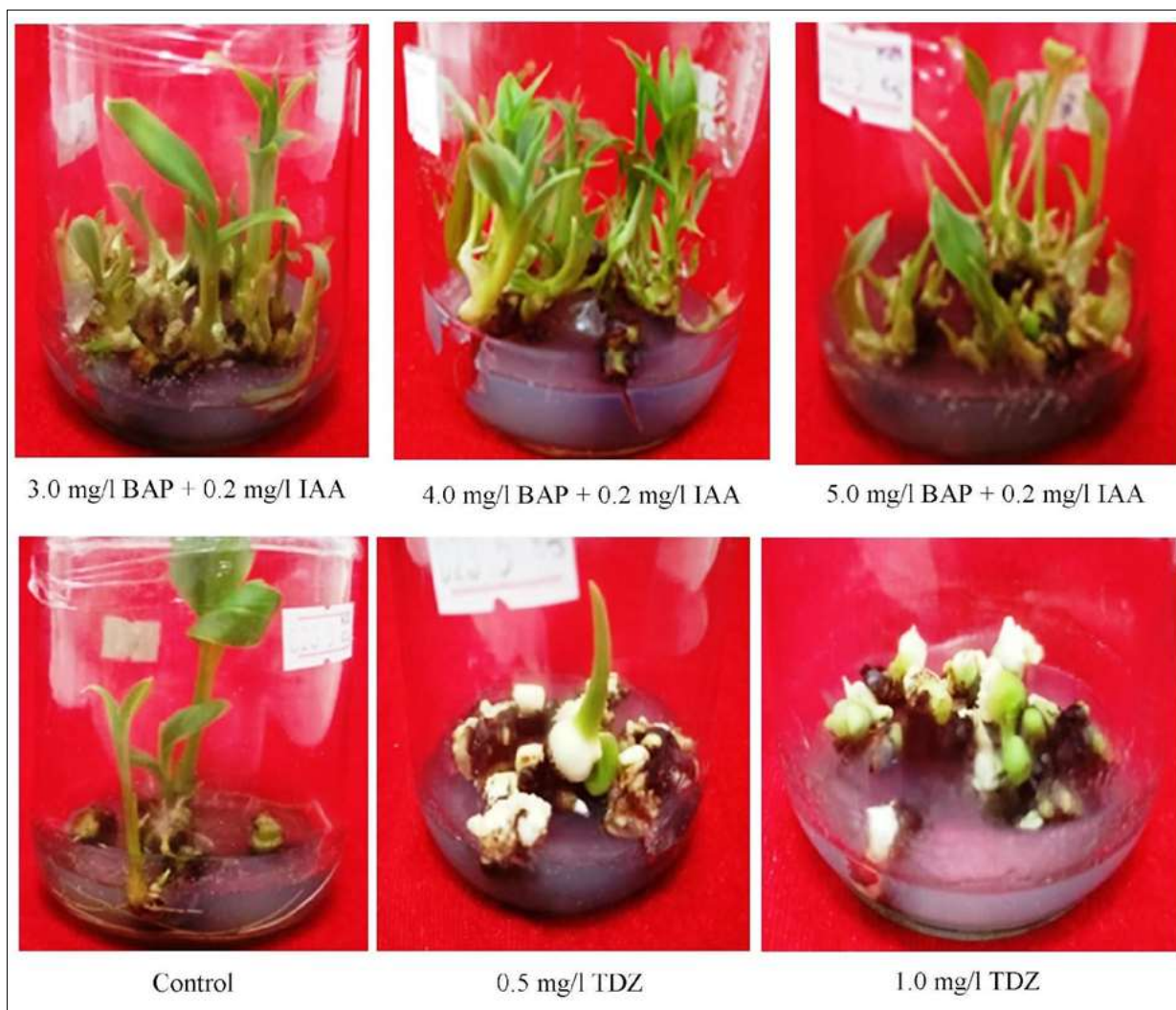
media treated with mT 6.0 mg/l (T_8) showed 27.9 shoots per culture. The explants cultivated on media fortified with TDZ 0.5 mg/l (T_5) produced the fewest number of shoots per explant (1.9 shoots) and was regarded as the least good treatment.

Explants cultured on the media supplemented with BAP 4.0 mg/l + IAA 0.2 mg/l (T_3) resulted highest mean number of shoots from cycle 1 to 5 in a consistent manner. Whereas, explants cultured in media supplemented with mT recorded higher number of shoots during the initial cycles but with the progression of the cycles, the number of shoots per culture

gradually decreased. However, the length and girth if the *in vitro* regenerated shoots were maximum in mT supplemented media over other cytokinins. Among the different cytokinins, the explants cultured on TDZ supplemented media were shown poor response with respect to the production of shoots. Media supplemented with TDZ produces small globular clump like structures without shoot elongation. Similar findings in banana were also reported by Ramachandran and Amrutha (2013) [20] and closely related to findings of Aman *et al.* (2018) [4], Dagnev *et al.* (2012) [7], Demissie (2013) [8], Oumar *et al.* (2018) [16].

Table 1: Effect of different growth hormones on number shoots per culture during different proliferation cycles in banana cv. Kovvur Bontha (ABB)

Treatments	Total number of shoots per culture						
	M ₁	C ₁	C ₂	C ₃	C ₄	C ₅	Average
CONTROL	2.60	2.33	4.53	4.55	3.07	3.44	3.42
BAP 3.0 mg/l + IAA 0.2mg/l	3.13	3.11	4.47	10.72	20.13	26.43	11.33
BAP 4.0 mg/l + IAA 0.2mg/l	3.00	7.33	10.33	13.67	28.20	33.42	15.99
BAP 5.0 mg/l + IAA 0.2mg/l	3.12	4.80	1.67	1.00	24.33	25.27	10.03
TDZ 0.5 mg/l	3.84	1.33	1.33	1.50	1.77	1.00	1.79
TDZ 1.0 mg/l	4.11	1.00	3.92	5.56	2.53	1.00	3.02
mT 4.0 mg/l	3.58	6.44	10.22	12.81	19.07	18.92	11.84
mT 6.0 mg/l	4.61	7.83	7.96	14.29	25.53	27.90	14.69
BAP 4.0 mg/l + Kn 1.0 mg/l+ IAA 0.2 mg/l	4.28	5.30	6.11	13.08	22.47	23.17	12.40
BAP 4.0 mg/l + Kn 2.0 mg/l+ IAA 0.2 mg/l	2.27	4.60	5.00	11.39	21.67	21.95	11.15
SE (m)	3.81	0.29	0.31	0.29	0.67	0.32	
CD (P=0.05)	NS	0.88	0.93	0.87	2.00	0.96	



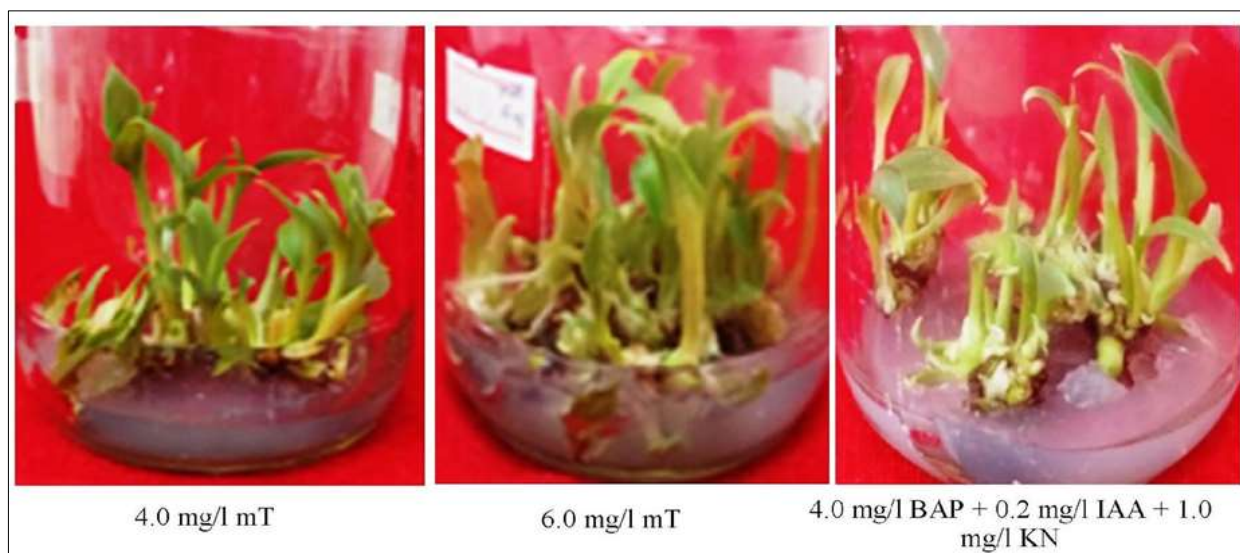


Fig 1: Effect of different plant growth regulators and their concentrations on *in vitro* shoot proliferation of banana cv. Kovvur Bontha (ABB, Bluggoe) in C₃ Cycle

Number of days taken for shoot initiation

Multiplication phase (M₁)

Days taken for shoot initiation in the multiplication phase showed statistically significant differences among the different treatments, as recorded in table 2. Explants cultured on media supplemented with mT 6.0 mg/l (T₈), had an early initiation (3.18 days), which was statistically on par with BAP 3.0 mg/l + IAA 0.2 mg/l (T₂), (3.37 days), BAP 4.0 mg/l + Kinetin 2.0 mg/l + IAA 0.2 mg/l (T₁₀), (3.41 days), BAP 4.0 mg/l + Kinetin 2.0 mg/l + IAA 0.2 mg/l (T₉), (3.44 days).

First subculture (C₁)

On perusal of data with respect to shoot initiation influenced by different concentrations and combinations of plant growth regulators, differed significantly at the end of the first subculture cycle. Among the different treatments evaluated (table 2), apical meristems cultured on the media enriched with MT 4.0 mg/l (T₇) recorded significantly early shoot response (5.0 days) followed by BAP 3.0 mg/l + IAA 0.2 mg/l (T₂), (6.3 days). The explants cultured on the media supplemented with TDZ 1.0 mg/l (T₆) responded very lately after 19.0 days, which was undesired.

Second subculture (C₂)

On perusal of the data in the Table 2 statistically significant differences were recorded among the treatments. According to the results in 4.6, culture responded in 5.9 days when meristems grown on MS media fortified with BAP 3.0 mg/l + IAA 0.2 mg/l (T₂) which was statistically on par with mT 6.0 mg/l (T₈) (7.2 days), BAP 4.0 mg/l + IAA 0.2 mg/l (T₃), (7.5 days), mT 4.0 mg/l (T₇), (7.6 days), control (T₁), (7.6 days), BAP 4.0 mg/l + Kinetin 2.0 mg/l + IAA 0.2 mg/l (T₁₀), (8.11 days), BAP 5.0 mg/l + IAA 0.2 mg/l (T₄), (8.1 days) followed by explants cultured on media fortified with BAP 4.0 mg/l + Kinetin 1.0 mg/l + IAA 0.2 mg/l (T₉), (9.0 days). The explants cultured on TDZ 0.5 mg/l (T₅) had taken the longest time (21.7 days).

Third subculture (C₃)

In the third subculture (C₃), significant differences were seen across all treatments for days taken for shoot initiation of banana explants (Table 2). Among the treatments, the apical

meristems cultured on the media supplied with BAP 3.0 mg/l + IAA 0.2 mg/l (T₂) leads to earliest shoot initiation (5.60 days) which was statistically on par with mT 4.0 mg/l (T₇), BAP 4.0 mg/l + IAA 0.2 mg/l (T₃), BAP 4.0 mg/l + Kinetin 1.0 mg/l + IAA 0.2 mg/l (T₉), BAP 4.0 mg/l + Kinetin 2.0 mg/l + IAA 0.2 mg/l (T₁₀), (6.0, 6.3, 6.4, 6.6 days respectively) followed by the apical meristems cultured on the media devoid of growth regulators *i.e.*, control (T₁) 7.5 days. The explants cultured on TDZ 0.5 mg/l (T₅) had a substantially higher number of days (20.7 days) for shoot initiation than the other treatments.

Fourth subculture (C₄)

Apical meristems cultured on media supplemented with BAP 3.0 mg/l + IAA 0.2 mg/l (T₂) had a significantly shorter shoot initiation time (5.5 days) which was statistically on par with mT 4.0 mg/l (T₇), mT 6.0 mg/l (T₈), BAP 4.0 mg/l + IAA 0.2 mg/l (T₃), BAP 5.0 mg/l + IAA 0.2 mg/l (T₄), BAP 4.0 mg/l + Kinetin 2.0 mg/l + IAA 0.2 mg/l (T₁₀), BAP 4.0 mg/l + Kinetin 1.0 mg/l + IAA 0.2 mg/l (T₉) had shown (6.0, 6.0, 6.1, 6.2, 6.3, 6.4 days respectively). These were followed by the explants cultured on media without growth regulators had shown 7.0 days. When the explants cultured on TDZ 0.5 mg/l (T₅) it was observed that delayed initiation of 16.0 days were taken for shoot initiation and was unsuitable for culture.

Fifth subculture (C₅)

It was evident from the data presented in Table 2, that the early shoot initiation was observed within 5.1 days when the explants were developed on media supplemented with BAP 3.0 mg/l + IAA 0.2 mg/l (T₂) which was found to be statistically on par with the meristems cultured on mT 6.0 mg/l (T₈), BAP 4.0 mg/l + IAA 0.2 mg/l (T₃), mT 4.0 mg/l (T₇) recorded 5.20, 5.25, 5.38 days and was followed by the explants supplemented with BAP 4.0 mg/l + Kinetin 2.0 mg/l + IAA 0.2 mg/l (T₁₀) 6.19 days. TDZ 0.5 mg/l (T₅) was the least effective treatment, in which explants resulting in a delayed shoot initiation (19.66 days) compared to all other treatments which was regarded unfavorable for culture.

Early shoot initiation was observed when the explants were developed on media supplemented with BAP 3.0 mg/l + IAA 0.2 mg/l (T₂). This might be due to the action of BAP in

promoting cell division and presence of endogenous auxin in the explant itself which can help shoot initiation. Similar findings were reported by Ferdousa *et al.* (2015) [10], Rabbani

et al., 1996 [18]; Khanam *et al.*, 1996 [12] but are in contradictory to the findings of Mangalore *et al.* (2021) [14] in banana.

Table 2: Effect of different growth hormones on number of days taken for shoot initiation during different proliferation cycles in banana cv. Kovvur Bontha (ABB)

Treatments	No. of days taken for shoot initiation						
	M ₁	C ₁	C ₂	C ₃	C ₄	C ₅	Average
CONTROL	6.17	7.00	7.61	7.49	7.00	8.66	7.16
BAP 3.0 mg/l + IAA 0.2mg/l	3.37	6.29	5.94	5.60	5.53	5.14	5.32
BAP 4.0 mg/l + IAA 0.2mg/l	5.18	6.66	7.50	6.33	6.10	5.25	6.17
BAP 5.0 mg/l + IAA 0.2mg/l	5.41	6.38	8.22	7.50	6.16	6.25	7.82
TDZ 0.5 mg/l	5.37	11.38	21.66	20.66	16.01	19.66	15.80
TDZ 1.0 mg/l	6.23	19.00	16.66	11.50	11.16	18.33	13.82
MT 4.0 mg/l	4.85	4.99	7.60	6.01	6.00	5.38	5.81
MT 6.0 mg/l	3.18	6.33	7.24	8.05	6.00	5.20	6.00
BAP 4.0 mg/l + Kn 1.0 mg/l+ IAA 0.2 mg/l	3.44	8.75	8.97	6.41	6.40	7.30	6.88
BAP 4.0 mg/l + Kn 2.0 mg/l+ IAA 0.2 mg/l	3.41	6.50	8.11	6.55	6.26	6.19	6.17
SE (m)	0.13	0.39	0.77	0.43	0.36	0.25	
CD (P=0.05)	0.39	1.18	2.31	1.28	1.07	0.75	

Rate of shoot multiplication

As per the data envisaged in table.3, explants cultured in MS media supplemented with BAP 4.0 mg/l + IAA 0.2 mg/l (T₃) was recorded highest average rate of shoot multiplication (2.31) preceded by mT 6.0 mg/l (2.25) which was statistically superior over all other treatments. Media devoid of growth regulators, and supplemented with TDZ had shown lowest rate of shoot multiplication and found not suitable for commercial production of banana cv. Kovvur Bontha (ABB). The most important phase in creating a successful micro-propagation protocol, and the step that has the biggest impact on the protocol's outcome, is shoot multiplication. It is interesting note from the above results on rate of shoot proliferation and number of shoots per explant, that these two parameters were inter dependent *i.e.* wherever the rate of shoot proliferation was higher, the number of shoots per explant was higher and *vice versa*. Shoot proliferation is mostly depends on number of factors like genotype, growth regulators, gelling agents and culture media composition.

Cytokinins such as BAP, mT, thiazuron (TDZ), and kinetin (KN) are used for the growth of axillary buds, and shoot multiplication. At present, in all the cycles (M₁ to C₅), media supplemented with TDZ and in the control cultures without any cytokinin supplementation were found to record the lowest number of shoots per culture and poor rate of multiplication. However, the rate of shoot proliferation was at the highest in BAP among the cytokinins used for supplementation in the present study, since it registered the maximum values. The other two *viz.*, mT and BAP +Kn were found to register lesser rates of shoot proliferation compared to BAP 4.0 mg/l + IAA 0.2 mg/l supplementation. Significant differences in respect of proliferation rate and number of shoots per explant due to cytokinins supplements were also registered by Abeyaretne and Lathiff (2002) [1], Deo and Pradhan (2017) [9], Bairu *et al.* (2008) [5], Bohra *et al.* (2014a) [6], Aman *et al.* (2018) [4], Siva Kumar and Visalakshi (2020) [21] in banana.

Table 3: Effect of different growth hormones on rate of shoot multiplication during different proliferation cycles in banana cv. Kovvur Bontha (ABB)

Treatments	Rate of shoot multiplication						
	M ₁	C ₁	C ₂	C ₃	C ₄	C ₅	Average
Control	1.27	1.24	1.37	1.09	1.00	1.06	1.17
BAP 3mg/l + IAA 0.2mg/l	1.59	1.19	1.40	1.79	2.22	2.46	1.78
BAP 4mg/l + IAA 0.2mg/l	1.60	1.92	2.08	2.16	2.91	3.11	2.30
BAP 5mg/l + IAA 0.2mg/l	1.34	1.67	1.25	1.00	2.47	2.13	1.64
TDZ 0.5mg/l	1.50	1.00	1.00	1.05	1.11	1.00	1.11
TDZ 1mg/l	1.79	1.00	1.25	1.12	1.17	1.06	1.23
MT 4mg/l	1.68	1.85	1.99	1.84	1.95	1.95	1.88
MT 6mg/l	1.95	1.98	1.77	2.20	2.62	2.96	2.25
BAP 4mg/l, Kn 1mg/l, and IAA 0.2mg/l	1.90	1.70	1.56	2.04	2.45	2.65	2.05
BAP 4mg/l, Kn 2mg/l, and IAA 0.2mg/l	1.22	1.62	1.52	1.95	2.31	2.60	1.87
SE (m)	0.12	0.08	0.10	0.072	0.13	0.11	
CD (P=0.05)	0.36	0.23	0.30	0.215	0.40	0.32	

Conclusion

The experiment concluded that, MS media supplemented with BAP 4.0 mg/l and IAA 0.2 mg/l produced the highest number of shoots per culture (16.0) with maximum rate of shoot proliferation (2.3) in minimum number of days (5.3 days) over all other cytokinins and their concentrations. Here, we

are reporting a very simple, economical, rapidly multiplying, and highly reproducible protocol for large scale production.

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