

## REGULATORY MECHANISMS OF PLANT GROWTH PROMOTING HORMONES AFFECTING REGENERATION POTENTIAL OF BANANA (*MUSA* SPP.)

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### ABSTRACT

This study investigates the regulatory mechanisms of hormones in banana (*Musa* spp.) regeneration. Benzylaminopurine (BAP) and Indole-3-acetic acid (IAA) were used. Study was conducted at the Plant Tissue Culture Laboratory, Agriculture Research Center, Tandojam. A total of 30 explants were cultured on basal medium supplemented with concentrations of BAP and IAA. BAP using varying compositions (SM-I to SM-VII), while IAA using different compositions (RM-I to RM-VII). The observation on survival rates, days to initiate shoots, total number of shoots, shoot length, and number of leaves was intimately observed. Regenerated shoots were then subjected to rooting media with varying IAA and sugar concentrations for root induction. Both BAP and IAA showed significant outcomes on shoot and root development. Growth media implied with MS-IV and MS-V concentrations of BAP effectively promoted shoot regeneration and elongation exhibiting the highest rates. Similarly, IAA-enriched concentrations influenced different aspects of growth, whereas RM-V demonstrated the longest roots and RM-II and RM-VII facilitated rapid shoot and root initiation. This knowledge is essential for sustainable agriculture, especially in the context of preserving genetic diversity and ensuring the availability of high-quality planting material of banana cultivations. Main objective of study are (1) To observe the regeneration and proliferation potential of banana through somatic tissues (2) To obtain the mass clonal production of banana through somatic tissues.

**Keywords:** Benzylaminopurine, regeneration, Indole-3-acetic acid, rapid shoot and root initiation.

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### INTRODUCTION

Banana (*Musa Cavendish* L.) possess a crucial position as one of the world's primary subsistence food crops. Widely cultivated in tropical and sub-tropical regions of the world, it also serves as a vital staple for millions in developing nations. Renowned for its exceptional nutritional profile, banana provides a unique blend of energy, along with essential elements for tissue-building, proteins, vitamins, and minerals. Moreover, it also has as a beneficial dietary option for individuals grappling with intestinal disorders like constipation, diarrhea, and dysentery (Roux *et al.*, 2001).

In Pakistan, banana stands as the second-largest fruit crop. The country's total cultivated area spans approximately 30 thousand hectares yielding an annual production of 134 thousand tons (FAOSTAT, 2014). Among the provinces, Sindh leads in banana production, boasting an impressive 25.4 thousand hectares and a total output of 112.9 thousand tons. Conversely, cultivation in the remaining provinces of Punjab, KPK, and Balochistan remains marginal (Al-Amin *et al.*, 2009).

The challenges faced by banana farmers encompass low yields and the prevalence of diseases, particularly Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cubense* (Njiguna *et al.*, 2008). Pest-related losses alone account for 30-80% of banana crop damage, which dependent on the specific variety. BBTV has led to significant losses across country (Pakistan), particularly in Sindh. This prompted a shift to other crops like sugarcane and cotton, but these alternatives failed to return banana's returns. Consequently, efforts focused on acquiring disease-free planting materials for replanting of plants on previously affected fields (Narayanamoorthy *et al.*, 2013).

Including different concentrations on shoot and root development of banana. Bananas significantly contribute to energy metabolism, nervous system function, and healthy digestion (Aprifel, 2015). The propagation of banana however, Conventional methods, however, introduce challenges like disease transmission, low yields, slow sucker multiplication rates, and genetic material preservation issues (Hussein, 2012). To counter these challenges, in vitro propagation through tissue culture has gained providing disease free planting material and mass propagation potential (Ali *et al.*, 2011).

As global demand for fruit, the widespread adoption of in vitro propagated banana can address these challenges and provide economically valuable commercial varieties (Ortiz *et al.*, 2014). Banana, rooted in the genetic intersection of *M. acuminata* and *M. balbisiana*, is among the oldest known fruits. Its significance is pronounced

across developing nations, where it contributes substantially to nutrition, trade, and income. With global banana yields reaching around 148 million tons in 2016, there's a growing need to leverage advanced techniques like plant tissue culture for germplasm improvement (Khaskheli *et al.*, 2021).

To overcome these challenges, the integration of Plant Cell and Tissue Culture techniques emerges as a viable solution for germplasm enhancement. Conventional methods encounter obstacles like polyploidy variation, selflessness, and slow sucker multiplication rates. *In-vitro* multiplication can mitigate pathogen spread and offer faster propagation rates (Khaskheli *et al.*, 2021).

## MATERIALS AND METHODS

### Plant material collection

The experiment was conducted at the Agriculture Research Center Tandojam (ARC), specifically at the Plant Disease Research Institute. Young suckers of banana (*Willium*) were collected as explants from mother plant source from the Experimental Farm of ARC Tandojam, Pakistan.

### Surface sterilization of explants

The collected explants were immediately rinsed under running tap water. Subsequently, they were subjected to sterilization using a solution of 70% ethanol or sodium hypochlorite (NaOCl), along with a few drops of Tween-20. The explants were then thoroughly washed with sterilized distilled water under a laminar airflow cabinet.

### Preparation of nutrient media

For the preparation of nutrient media, method outlined by Murashige and Skoog (MS) was followed. The MS basal media containing macro and micro elements, as well as vitamins was used. Additionally, growth hormones were introduced in various combinations. The pH of the medium was adjusted to 5.8 before autoclaving at 121°C for 20 minutes.

### Incubation conditions

Culture bottles were placed in a growth/culture room with a temperature maintained at 25±2°C. The photoperiod was in accordance with the requirements of the experiment.

### Composition of shoot proliferation media

SM-I = MS-Media + BAP 0.0 mgL<sup>-1</sup> + 30g Sucrose  
SM-II = MS-Media + BAP 0.5 mgL<sup>-1</sup> + 30g Sucrose  
SM-III = MS-Media + BAP 1.0 mgL<sup>-1</sup> + 30g Sucrose  
SM-IV = MS-Media + BAP 1.5 mgL<sup>-1</sup> + 30g Sucrose  
SM-V = MS-Media + BAP 2.0 mgL<sup>-1</sup> + 30g Sucrose  
SM-VI = MS-Media + BAP 3.0 mgL<sup>-1</sup> + 30g Sucrose  
SM-VII = MS-Media + BAP 3.5 mgL<sup>-1</sup> + 30g Sucrose

### Composition of root induction media

RM-I = MS-Media + IAA 0.0 mgL<sup>-1</sup> + 30g Sucrose  
RM-II = MS-Media + IAA 0.5 mgL<sup>-1</sup> + 30g Sucrose  
RM-III = MS-Media + IAA 1.0 mgL<sup>-1</sup> + 30g Sucrose  
RM-IV = MS-Media + IAA 1.5 mgL<sup>-1</sup> + 30g Sucrose  
RM-V = MS-Media + IAA 2.0 mgL<sup>-1</sup> + 30g Sucrose  
RM-VI = MS-Media + IAA 3.0 mgL<sup>-1</sup> + 30g Sucrose  
RM-VII = MS-Media + IAA 3.5 mgL<sup>-1</sup> + 30g Sucrose

### Experimental designs

The experimental procedure followed by using Completely Randomized Design (CRD). The micro-propagation technique was employed for tissue culturing of banana. Sterilized explants were cultured on Murashige and Skoog (1962) Basal medium. Around 20 explants were placed on each Basal medium supplemented with different concentration combinations of BAP + Sugar mg L<sup>-1</sup> for shoot induction.

### Observations recorded

Observations were recorded for various parameters, including days to initiate shoots bottle<sup>-1</sup>, total number of shoots bottle<sup>-1</sup>, length of shoots bottle<sup>-1</sup> (cm) and total number of leaves bottle<sup>-1</sup>. Furthermore, regenerated shoots were dissected and transferred to rooting media with varying concentrations of combinations of (IAA + Sugar mgL<sup>-1</sup>) for root induction. Observations were made for days to initiate roots bottle<sup>-1</sup>, total number of roots bottle<sup>-1</sup> and length of roots bottle<sup>-1</sup> (cm), survival rate (%).

### Statistical analysis

The experimental data was collected and subjected to a factorial design analysis of variance (ANOVA) using linear models of statistics. This analysis aimed to identify statistical differences among different concentrations of plant growth regulators. The analysis was conducted using the Student Edition of Statistix (SWX), Version 8.1 (Analytical Software, 2005).

## RESULTS

### Days to initiate shoots (bottle<sup>-1</sup>)

Table 1 shows the dataset provided furnishes insights into the duration required for shoot initiation in banana tissue culture experiments across different media concentrations. With each concentration subjected to three replications, the findings shed light on the initiation process. Notably, SM-III emerges as the most expedient in initiating shoots, with an average of 12.00 days across its replications of 13, 12 and 11 days. Following suit, SM-IV demonstrates a swift initiation period at 12.66 days, reflecting replications of 14, 11 and 13 days. Comparably, SM-V reports an average initiation time of 14.66 days, as its replications span 14, 15 and 15 days. In contrast, SM-VI exhibits a lengthier initiation phase, requiring an average of 20.33 days and its replications of 25, 20 and 16 days. SM-II indicates a mean initiation period of 17.00 days, showcasing variability within its replications. These observations spotlight the substantial impact of media concentrations on the pace of shoot initiation in banana tissue culture, underscoring the pivotal role of strategic concentration selection for efficient and timely shoot initiation processes.



Fig. 1. Initiation of shoots under Different treatment of BAP.

- initiation after two weeks of culturing
- Shoot initiation after three weeks of culturing
- Shoots starting
- Multiple shoots regenerated

### Shoot length (cm) bottle<sup>-1</sup>

In Table 2 the experiment investigating shoot length variations in response to different concentrations of BAP for banana tissue culture revealed notable patterns. Among the tested concentrations, Media Concentrations MS-III and MS-IV displayed the highest shoot lengths, with means of 6.2000 cm and 5.9333 cm, respectively. These concentrations could be considered optimal for promoting longer shoot growth. On the other hand, Media Concentrations MS-VII exhibited the lowest shoot lengths, with a mean of approximately 3.2 cm, suggesting relatively restrained growth. In terms of the most effective concentration, Media Concentration MS-III emerged as the one yielding the highest mean shoot length, while Media Concentration MS-VII resulted in the lowest. This distinction in shoot lengths among the concentrations underscores the significance of selecting appropriate growth regulator concentrations for achieving desirable shoot elongation in banana tissue culture.

### Number of shoots (bottle<sup>-1</sup>)

The outcomes of shoot number in banana tissue culture experiments, utilizing different media concentrations with varying levels of BAP (benzylaminopurine), are presented in the provided Table 3. The results reflect the shoot number achieved through three separate replications for each media concentration. Among the BAP treatments, MS-V demonstrated the highest mean shoot number, with an average value of 5.66. This concentration exhibited consistent results across its replications, with recorded shoot numbers of 6, 5 and 6. Conversely, MS-I (control)

displayed the lowest mean shoot number of 0.00, reflecting no discernible shoot regeneration across its replications. The influence of BAP concentrations on shoot numbers is evident in the trend of increasing shoot numbers with higher BAP concentrations. This observation is supported by the ascending mean shoot numbers observed from MS-II to MS-V. Notably, MS-III and MS-IV shared a mean shoot number of 3.66, signifying effective shoot regeneration under these concentrations. The findings underscore the impact of BAP concentrations on shoot number outcomes in banana tissue culture, highlighting the importance of suitable concentration selection for optimal shoot proliferation.



Fig. 2. Multiple shoot regeneration under different treatment of BAP.

#### Number of leaves (bottle<sup>-1</sup>)

The provided Table 4 outlines the results of banana tissue culture experiments focused on determining the number of leaves under different media concentrations. Each concentration was subjected to three replications to assess its impact on leaf production. Among the examined concentrations, SM-III emerged as the most conducive for leaf growth, boasting the highest mean number of leaves at 14.33. This observation is supported by consistent replication results of 16, 13, and 14 leaves. Similarly, SM-IV demonstrated notable leaf proliferation, displaying a mean of 16.00 leaves across its replications of 13, 18, and 17 leaves. SM-V also showcased a substantial mean number of leaves at 14.00, with replications yielding 15, 13 and 14 leaves. On the other hand, SM-VII exhibited the lowest mean number of leaves at 10.33, as a result of its replication values of 11, 14 and 6 leaves. SM-VI occupied an intermediary position with an average leaf count of 13.25, aligning it between the more and less productive concentrations. The data underscores the critical role of media concentrations in influencing leaf production in banana tissue culture, emphasizing the necessity of strategic concentration selection to optimize leaf development outcomes.

#### Days to initiate roots (bottle<sup>-1</sup>)

The dataset provided Table 5 offers insights into the timeline for root initiation within banana tissue culture experiments, particularly under varying media concentrations supplemented with IAA (indole-3-acetic acid). Each concentration underwent rigorous testing across three replications to ascertain the initiation period. Notably, RM-II and RM-VII stand out as the media concentrations facilitating the swiftest root initiation, both with a mean initiation time of 18.66 days. The former is characterized by replications of 16, 18, and 22 days, while the latter displays replication durations of 20, 19 and 17 days.

Meanwhile, RM-III emerges as a close contender, with a mean initiation time of 13.33 days supported by replications of 14, 13 and 13 days. In a similar vein, RM-IV, RM-V, and RM-VI showcase average initiation times ranging from 12.66 to 14.66 days, with corresponding replications. These findings accentuate the interplay between IAA-based media concentrations and the timing of root initiation in banana tissue culture.



Fig.3. Initiation root proliferation under different treatment of IAA.

Table 1. Days to initiate shoots (bottle<sup>-1</sup>) of banana regenerated on MS-Basel media supplemented with different concentration of BAP.

Media Concentrations	Replications			Mean
	R-I	R-II	R-III	
SM-I	0.00	0.00	0.00	0.00 g
SM-II	3.4	3.0	3.6	3.33 ef
SM-III	5.6	7.8	5.2	6.20 a
SM-IV	5.9	5.4	6.5	5.93 b
SM-V	4.8	5.4	4.8	5.00 c
SM-VI	3.0	4.8	5.7	4.5 d
SM-VII	3.2	2.9	3.5	3.2 e
SE Mean = 2.9589      CV = 1.2795      Grand Mean = 8.718254 SD = 7.8286      LSD (5%) = 0.5966				

Table 2. Shoot length (cm) of banana regenerated on MS-Basel media supplemented with different concentration of BAP.

Media Concentrations	Replications			Mean
	R-I	R-II	R-III	
SM-I	0	0	0	0.000 f
SM-II	14	19	18	17.00 bc
SM-III	13	12	11	12.00 de
SM-IV	14	11	13	12.66 d
SM-V	14	15	15	14.66 c
SM-VI	25	20	16	20.33 ab
SM-VII	28	28	18	24.66 a
SE Mean = 9.1099      CV = 2.145      Grand Mean = 28.95238 SD = 24.1026      LSD (5%) = 5.6034				

Table 3. Number of shoots (bottle<sup>-1</sup>) of banana regenerated on MS-Basel media supplemented with different concentration of BAP.

Media Concentrations	Replications			Mean
	R-I	R-II	R-III	
SM-I	0	0	0	0.00 e
SM-II	1	3	2	2.00 d
SM-III	2	4	5	3.66 b
SM-IV	4	3	4	3.66 b
SM-V	6	5	6	5.66 a
SM-VI	3	3	4	3.33 bc
SM-VII	2	3	2	2.33 bc
SE=Mean = 2.1069      CV = 1.4299SE      Grand Mean = 6.396825 SD = 5.5743      LSD (5%) = 2.533				

Table 4. Number of leaves (bottle<sup>-1</sup>) of banana regenerated on MS-Basel media supplemented with different concentration of BAP.

Media Concentrations	Replications			Mean
	R-I	R-II	R-III	
SM-I	0	0	0	0.00 f
SM-II	10	9	8	9.00 e
SM-III	16	13	14	14.33 b
SM-IV	13	18	17	16.00 a
SM-V	15	13	14	14.00 b
SM-VI	15	20	11	13.25 bc
SM-VII	11	14	6	10.33 d
SE Mean = 10.108      CV = 7.0322      Grand Mean =24.45238 SD = 26.7436      LSD (5%) = 2.080				

Table 5. Days to initiate roots (bottle<sup>-1</sup>) of banana regenerated on MS-Basel media supplemented with different concentration of IAA.

Media Concentrations	Replications			Mean
	R-I	R-II	R-III	
RM-I	0	0	0	0.00 f
RM-II	11	10	11	10.1 e
RM-III	14	13	13	13.33 c
RM-IV	12	11	15	12.66 d
RM-V	15	15	14	14.66 b
RM-VI	17	14	11	14.00 b
RM-VII	20	19	17	18.66 a
SE Mean = 7.3716      CV = 2.145      Grand Mean = 26.28571 SD = 19.5032      LSD (5%) = 3.3533				

Table 6. Root length (cm) of banana regenerated on MS-Basel media supplemented with different concentration of IAA.

Media Concentrations	Replications			Mean
	R-I	R-II	R-III	
RM-I	0.00	0.00	0.00	0.00 g
RM-II	2.3	2.5	3.0	2.66 f
RM-III	3.5	3.3	3.5	3.43 d
RM-IV	4.1	4.5	3.9	4.16 b
RM-V	5.2	5.5	4.7	5.13 a
RM-VI	3.8	3.5	4.5	3.93 c
RM-VII	2.4	2.2	3.9	2.83 e
SE Mean = 1.9012      CV = 0.7430      Grand Mean =6.840476 SD=5.0304      LSD (5%) = 2.165				

Table 7. Number of roots (bottle<sup>-1</sup>) of banana regenerated on MS-Basel media supplemented with different concentration of IAA.

Media Concentrations	Replications			Mean
	R-I	R-II	R-III	
RM-I	0	0	0	0.00 g
RM-II	5	7	7	6.33 d
RM-III	7	11	11	9.66 a
RM-IV	8	10	4	7.33 b
RM-V	7	8	6	7.00 c
RM-VI	7	5	6	6.00 d
RM-VII	4	9	4	5.66 f
SE Mean = 4.524      CV = 4.2896      Grand Mean = 13.2698 SD=11.9694      LSD (5%) = 2.145				

Table 8. Survival rate (%) of banana regenerated on MS-Basel media supplemented with different concentration of BAP.

Media Concentrations	Replications			Mean
	R-I	R-II	R-III	
SM-I	0	0	0	0.00 c
SM-II	63	65	65	64.33ab
SM-III	69	73	78	73.33 ab
SM-IV	81	86	91	86 a
SM-V	93	87	95	91.66a
SM-VI	74	70	72	72 ab
SM-VII	58	57	61	58.66 ab
SE Mean = 11.49467      CV = 47.72667      Grand Mean = 63.71429 SD = 30.412      LSD (5%) = 3.0809				

### Root length (cm) bottle<sup>-1</sup>

In Table 6 the dataset presented provides valuable insights into the root length observed in banana tissue culture experiments conducted under diverse media concentrations enriched with IAA (indole-3-acetic acid). By subjecting each concentration to three separate replications, researchers sought to comprehend the impact of these variations on root development. Significantly, RM-V emerges as the most conducive environment for robust root growth, displaying the longest mean root length at 5.13 cm. This achievement is mirrored by consistently high individual replications of 5.2, 5.5 cm and 4.7 cm. In a closely contested range, RM-III, RM-IV and RM-VI exhibit mean root lengths ranging from 3.43 cm to 4.16 cm. Both RM-II and RM-VII, while presenting relatively shorter mean root lengths at 2.66 cm and 2.83 cm, respectively, complete the spectrum. These outcomes underscore the potent interplay between media concentrations infused with IAA and the growth of roots in banana tissue culture.

### Number of roots (bottle<sup>-1</sup>)

In Table 7 the dataset provided presents a comprehensive insight into the number of roots observed within banana tissue culture experiments conducted under varying media concentrations supplemented with IAA (indole-3-acetic acid). Through meticulous replication, each concentration was meticulously evaluated for its effect on root production. Remarkably, RM-III stands out as the most prolific in root generation, exhibiting the highest mean number of roots at 9.66. This outcome is supported by robust individual replications of 7, 11, and 11 roots. Following closely, RM-IV demonstrates a substantial mean root count of 7.33, thanks to replications of 8, 10, and 4 roots. On a similar note, RM-II, RM-V, RM-VI and RM-VII showcase mean root counts ranging from 5.66 to 7.33. These results illuminate the intricate interplay between IAA-containing media concentrations and the root development process within banana tissue culture.

### Survival rate (%)

In Table 8 the dataset provided sheds light on the survival rates observed in banana tissue culture experiments conducted under different media concentrations. Through rigorous replication, these experiments aimed to understand the impact of varying concentrations on the survival of banana cultures. Notably, MS-V emerges as the most conducive environment for survival, boasting a substantial mean survival rate of 91.66. This outcome is mirrored by robust individual replications of 93, 87 and 95. MS-IV closely follows with a notable mean survival rate of 86, achieved through replications of 81, 86 and 91. Similarly, MS-III and MS-VI exhibit mean survival rates of 73.33 and 72 respectively, while MS-II and MS-VII present lower mean survival rates of 64.33 and 58.66 respectively. The nuanced variations in survival rates underscore the intricate interplay between media concentrations and survival outcomes in banana tissue culture.

## DISCUSSION

The study conducted by Kandha *et al.* (2021) on the valuable comparison with research endeavors of different BAP concentration. Both studies share a common objective of optimizing tissue culture techniques for effective banana propagation. Kandha's investigated the effulgence of 'Grand Naine' cultivar using rhizome and sucker explants aligns with specially focus on growth parameters and growth-promoting substances. Notably, studies highlighted the pivotal influence of growth-promoting substances specifically BAP and IAA on tissue culture outcomes. Kandha's findings, alongside with specific combinations of BAP and IAA concentrations contribute to successful shoot and root regeneration a finding of paramount importance for tissue culture optimization.

Furthermore, Kandha's study introduces the novel element of chitosan supplementation to enhance explant growth. This innovative approach diverges from the present research which primarily centers on the effects of BAP and IAA. Additionally, both studies place a strong emphasis on the practical implications of their findings. Kandha's successful greenhouse acclimatized underscores the relevance of achieving high post-tissue culture survival rates a goal shared by our research. Ultimately, the convergence of objectives and methods between these studies underscores the collective endeavor to enhance tissue culture techniques for practical application in banana cultivation. The insights provided by both studies contribute significantly to the advancement of optimized tissue culture protocols with Kandha's incorporation of chitosan offering a novel avenue for consideration in future research and practical applications.

Shah *et al.* (2020) further explored the impact of BAP and IAA concentrations on banana shoot proliferation and root induction. Their study reinforced the importance of optimizing growth regulator combinations, echoing our research and highlighting the significance of strategic concentrations. Paradhan and Deo's (2019) exploration into the effects of cytokinins and auxins on shoot proliferation and root induction expands the understanding of growth regulator influences. Their findings, similar to our research and others, underscore the necessity of precise growth regulator concentrations for different stages of in vitro propagation.

When comparing these studies to our work research focus on growth regulator optimization becomes apparent and emphasizing the pivotal role of these substances in successful banana tissue culture. The consistency in objectives methods and outcomes across these studies underscores the collective effort to refine tissue culture techniques for practical banana cultivation. Moreover, these studies linked with Horie *et al.* (2020) findings that reveals a common theme of tailored growth regulator concentrations for optimal results. This reinforces the universal importance of targeted growth regulator application in the pursuit of efficient and viable banana micro propagation protocols.

## Conclusions

The results presented the data a series of banana tissue culture experiments highlights the critical influence of media concentrations and growth-promoting substances on various growth parameters. Media concentrations, the number of shoots leaves and initiation times and intricate relationship between media components and growth outcomes. These findings underscore the significance of tailored media formulations for optimal shoot and root development in banana tissue culture, offering valuable insights for enhancing banana propagation techniques.

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