Optimization of Growth Condition for Micropropagation of Banana (*Musa spp*.) in a Temporary Immersion Bioreactor System

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Abstract
This study aimed to optimize growth conditions for micropropagation of banana (Musa spp.) using temporary immersion bioreactors (TIBs). The experiment evaluated the influence of two factors: feeding frequency and cytokinin concentration. Two feeding schedules were tested: 6 immersions per day and 8 immersions per day. The TIBs were submerged in the culture medium for a set duration during each immersion, with the remaining time allowing for gas exchange and drainage. The culture medium was supplemented with Benzylaminopurine (BAP), a plant growth regulator, at five different concentrations: 2.5 mg/L, 3.5 mg/L, 4.5 mg/L,5.5 mg/L and 6.5mg/L. BAP promotes shoot multiplication, a crucial aspect of micropropagation. All other culture conditions, such as light intensity, photoperiod, and medium composition, were kept constant throughout the experiment. Following an incubation period, banana microshoot growth parameters were assessed. The parameter taking is number of new shoots produced per initial explant. The results revealed that a BAP concentration of 4.5 mg/L yielded the most optimal growth for the banana microshoots. This suggests that 4.5 mg/L BAP effectively balances shoot proliferation without compromising shoot development. Furthermore, the experiment demonstrated a significant improvement in growth parameters when the feeding frequency was increased to 8 immersions per day compared to 6 immersions per day. This indicates that more frequent exposure to the culture medium enhances nutrient and oxygen availability, leading to better micropropagation outcomes. This study identified a combination of 4.5 mg/L BAP and a feeding schedule of 8 immersions per day as the optimal conditions for micropropagation of banana in TIBs. These findings can contribute to improved efficiency and productivity in banana micropropagation for commercial or research purposes.

Keywords: Micropropagation, Photoperiod, Incubation, Microshoots, Immersion.

Introduction

Tissue culture grows plants from tissue or cells in a lab, using techniques like shoot, meristem, callus culture, somatic embryogenesis, cell suspension, and protoplast cultures for banana propagation. Banana (*Musa spp.*) is a crucial food crop and major export, grown in 130 countries, and originating from Southern Asia (Ahmed *et al.*, 2014).

In vitro propagation, especially using Temporary Immersion Bioreactor Systems (TIBs) offers advantages over conventional methods, such as higher multiplication rates, disease-free plants, and faster growth. (Ikram-Ul-Haq & Dahot, 2007). TIBs, which use intermittent immersion in liquid nutrients, enhance propagation efficiency and facilitate disease-free cultivar production. This study highlights efficient banana propagation using TIBs. (Karule *et al.*, 2016; Gubbuk & Pekmezci, 2004).

Materials and Methods

Plant Materials: Young banana suckers were collected from the National Biotechnology Development Agency (NABDA) nursery. Explants were trimmed, washed, and sterilized.

Explant Preparation: Suckers were cleaned with plant detergent (Tween 20) and water, outer leaf sheaths were removed, and shoot tips were isolated. Explants were sterilized with ethanol, and NaOCl, and rinsed with sterile water.

Culture Establishment: Explants were cultured on modified Murashige and Skoog (MS) medium with BAP and IAA, incubated at 25°C under 16h light for 8 weeks. Subculturing occurred every 4 weeks.

Temporary Immersion Bioreactor System: TIBs filled with MS medium and various BAP concentrations were used. Two treatments, 6 and 8 immersions per day, ensured nutrient exposure. Shoots were transferred to rooting media and then acclimatized in nursery bags for 4 weeks.

Results and Discussion

ANOVA Results Interpretation:

Effect of Weeks: Significant variation in propagation (sum of squares: 3689.63) with a strong effect (F-value: 113.55) and a highly significant p-value (2.92e-52), indicating that different weeks significantly affect propagation rates.

Effect of Concentration: Substantial variation (sum of squares: 6196.85) with a very strong effect (F-value: 190.64) and a highly significant p-value (3.43e-68), showing that different concentration levels significantly influence propagation rates.

Effect of Cycles: Some variation (sum of squares: 193.03) with a strong effect (F-value: 23.75) and a significant p-value (3.45e-06), indicating that the number of cycles significantly affects propagation rates.

Effect of Replicate: Minimal variation (sum of squares: 5.02) with a weak effect (F-value: 0.31) and a non-significant p-value (0.735), meaning replication does not significantly affect propagation rates.

Residual: Represents unexplained variation within groups (sum of squares: 585.87, df: 72).

This study examined the impact of concentration levels, exposure times (weeks), cycles, and replicates on banana propagation rates. ANOVA results indicated that concentration and weeks significantly affect propagation, with high F-values and negligible p-values. Longer durations (weeks) and higher concentrations resulted in increased propagation rates, suggesting their critical

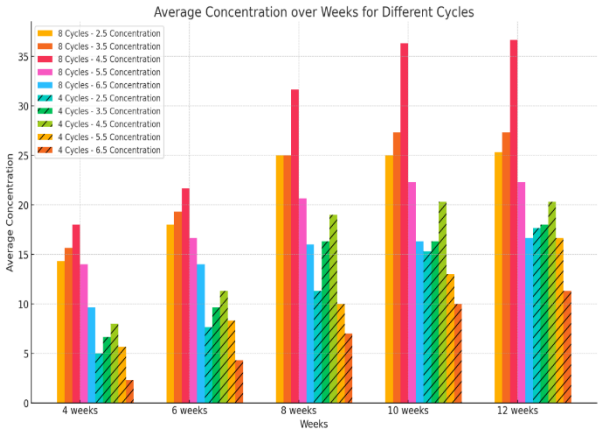
Table 1: Banana Shoot Propagation Data from TIBs: Considering BAP Concentration and Number of Cycles

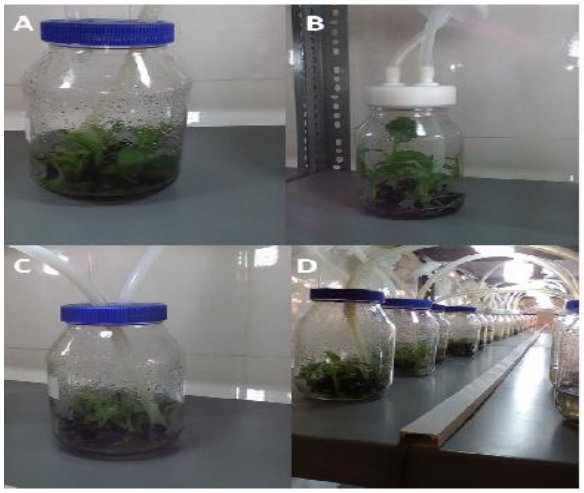
WEEKS	CONCENTRATION	2.5			3.5			4.5			5.5			6.5		
	Replicate/No of Cycles	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
4	8 CYCLES	12	14	17	13	15	19	18	17	19	15	10	17	10	10	9
	4 CYCLES	6	2	7	5	7	8	6	9	9	5	5	7	3	1	3
6	8 CYCLES	15	18	21	17	18	23	21	21	23	18	12	20	18	12	12
	4 CYCLES	8	6	9	9	9	11	10	11	13	8	8	9	5	3	5
8	8 CYCLES	20	26	29	23	23	29	28	32	35	22	17	23	18	14	16
	4 CYCLES	11	10	13	14	17	18	15	19	23	10	10	10	6	7	8
10	8 CYCLES	20	26	29	25	26	31	32	38	39	22	20	25	18	14	17
	4 CYCLES	15	13	18	14	17	18	16	20	25	12	13	14	10	8	12
12	8 CYCLES	20	27	29	25	26	31	32	38	40	22	20	25	19	14	17
	4 CYCLES	18	16	19	16	18	20	16	20	25	14	17	19	11	11	12

Table 2: ANOVA Table

Source	Sum of squares	dF	Mean square	F-Value	P-Value
C (WEEKS)	3689.6333	4	922.4083	113.5456	2.92e-52
C (CONCENT)	6196.8500	4	1549.2125	190.6375	3.43e-68
C (CYCLES)	193.0333	1	193.0333	23.7481	3.45e06
C (REPLICATE	5.0167	2	2.5083	0.3086	0.7352
Residual	585.8667	72	8.1370		

roles in growth, which is in agreement with Ahmed *et al.* (2014). The findings of Ikram-Ul-Haq and Dahot, M. U. (2007), also agree with our results where the number of cycles significantly influenced propagation, with more cycles yielding higher rates. Replicates did not significantly impact propagation, indicating consistency across trials. Residual variation was minimal. Overall, optimizing weeks, concentration, and cycles is essential for maximizing propagation rates. Future studies should explore the underlying mechanisms of these interactions for refined optimization strategies.







Conclusion

Optimizing weeks, concentration levels, and cycles significantly enhances banana propagation rates. Each factor independently affects propagation, with longer durations and higher concentrations yielding the best outcomes. Consistent results across replicates confirm reliability, emphasizing the importance of fine-tuning these parameters for efficient banana tissue culture propagation.

Some References

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