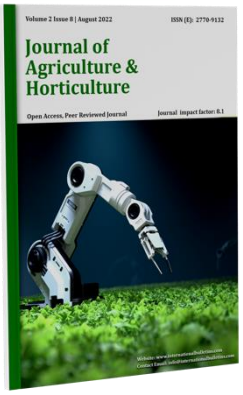

INTRODUCTION OF BANANA VARIETIES INTO CULTIVATION AND THE USE OF PHYTOHORMONES IN NUTRIENT MEDIA FOR CLONAL MICROPROPAGATION

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Abstract

This research presents the development of in vitro micropropagation protocols for banana (Musa spp.) varieties under controlled laboratory conditions in Uzbekistan. The study was conducted from 2022 to 2024 at the Biotechnology Laboratory of the Scientific Research Institute of Horticulture, Viticulture, and Winemaking named after Academician Mahmud Mirzayev. Explants were collected from greenhouse-grown banana plants and subjected to surface sterilization using sodium hypochlorite (NaOCl) at varying concentrations and exposure times.

The explants were cultured on different nutrient media—MS, DKW, WPM—supplemented with plant growth regulators, mainly BAP and IBA. Results showed that the survival and regeneration of banana explants are highly dependent on the sterilization conditions and the hormonal composition of the media. Enhanced MS medium combined with 2.5–4.0 mg/L BAP and 0.1 mg/L IBA provided the best outcomes in terms of shoot proliferation and elongation. Additionally, IBA was found to significantly improve root development during the acclimatization phase.

The findings offer a scientific basis for the mass propagation of disease-free banana planting materials and contribute to the advancement of banana cultivation practices in Uzbekistan's agro-climatic conditions.

Keywords: *Banana (Musa spp.), micropropagation, in vitro culture, BAP, IBA, sodium hypochlorite, tissue culture, Uzbekistan.*

Introduction

The increase in the world population and its growing demand for food requires the expansion of agricultural production, efficient use of agricultural land, and the development and implementation of new innovative technologies. Globally, bananas are cultivated on 5.97 million hectares of land, producing a total yield of 139.28 million tons. Of this total, 51.9% is produced in Asia and 23.9% in the Americas. India leads banana production with 36.6 million tons annually. China produces 11.7 million tons, Indonesia 9.3 million tons, and Nigeria 7.3 million tons. In the Americas, Ecuador and Brazil are the leading producers, with 7.2 and 6.8 million tons respectively each year (FAOstat, 2023).

In Uzbekistan, bananas are mostly grown in greenhouse conditions, but currently, only a few greenhouses are involved in banana cultivation. No scientific studies have yet been conducted in our country regarding the phenological development stages of banana plants and their resistance to heat and cold. It is essential to select promising banana varieties with

favorable agro-biological characteristics suitable for our climate and to develop appropriate technologies to achieve high yields.

Banana plant micropropagation under in vitro conditions was first studied by Berg and Bustamante (1974), who combined banana meristem shoot culture with thermotherapy to produce virus-free plants. Ma and Shii (1972) used shoot tips of Cavendish (AAA) bananas isolated from seedlings as a method for rapid multiplication of planting material resistant to Fusarium wilt. Since then, microclonal propagation of banana plants has been applied to a wide range of varieties (Vuylsteke, 1998) and has been used as a tool to maximize banana production, contributing to various aspects of banana tissue culture (Smith et al., 2005). In the early 1980s, the practice of acclimatizing and planting young banana seedlings in open fields was widely adopted in Taiwan and Jamaica (Hwang et al., 1984; Ogelsby & Griffis, 1986).

Materials and methods

This research was conducted from 2022 to 2024 at the Biotechnology Laboratory of the Scientific Research Institute of Horticulture, Viticulture, and Winemaking named after Academician Mahmud Mirzayev. The objective of the study was to establish protocols for the in vitro culture and micropropagation of banana (Musa spp.) cultivars under controlled laboratory conditions.

The initial plant material was collected from the protected cultivation area of the "Salar – Nosirov Murod Farmer Enterprise," located in Qibray District, Tashkent Region. Mother plants were selected from a greenhouse environment where healthy banana plants were being cultivated. Selected explants were transported to the laboratory's sterile facility, where they were thoroughly rinsed with distilled water to remove surface contaminants.

Following the initial wash, explants were surface-sterilized using sodium hypochlorite (NaClO) solutions at concentrations of 1.0% and 2.0% for different exposure durations of 10, 15, 20, and 25 minutes. After sterilization, the explants were carefully rinsed with sterile distilled water and cultured individually in test tubes containing solidified nutrient media.

For the initiation and multiplication phases, various culture media were tested, including Murashige & Skoog (MS), Driver & Kuniyuki Walnut (DKW), and Woody Plant Medium (WPM). These media were supplemented with different concentrations and combinations of plant growth regulators, primarily Indole-3-butyric acid (IBA) and 6-Benzylaminopurine (BAP), to evaluate their effects on shoot initiation and multiplication rates.

This study represents the first systematic attempt in Uzbekistan to develop micropropagation protocols for banana cultivars using tissue culture techniques. The findings aim to contribute to the establishment of a reliable method for the rapid, large-scale production of disease-free banana planting material suitable for local agro-climatic conditions.

Results and discussion

Plants cultivated in open field conditions are often contaminated with various microorganisms, which pose a significant challenge during the establishment of in vitro cultures. Therefore, explants must undergo proper disinfection prior to their transfer to sterile culture conditions. Goswami and Handique (2013) have provided valuable insights into the surface sterilization of the modified underground stem parts of banana plants.

Various sterilization procedures and chemical disinfectants have been proposed for use in multiple in vitro experiments. Among them, sodium hypochlorite (NaOCl) is the most widely used surface sterilizing agent for banana explants (Muhamad et al., 2004; Srangsam & Kanchanapoom, 2007; Farahani et al., 2008; AL-Amin et al., 2009). However, some studies have reported the use of low concentrations of mercuric chloride ($HgCl_2$) as an alternative to sodium hypochlorite. This approach was discussed by Molla et al. (2004), Titov et al. (2006),

and Goswami & Handique (2013), who observed its effectiveness in achieving higher levels of sterilization, albeit with associated toxicity concerns.

The process of micropropagation of banana cultivars under in vitro conditions begins with the introduction of vegetative plant parts into a sterile culture medium. This culture initiation stage is a critical starting point in the microclonal propagation technology. During this stage, virus-free, physiologically active, and regeneration-competent parts of the plant—primarily apical or axillary buds and meristematic tissues—are carefully selected as explants.

To eliminate microbial and pathogenic contaminants on the surface of the explants, they are treated with sodium hypochlorite (NaOCl) under carefully controlled conditions. Following sterilization, the clean explants are transferred to a nutrient medium based on the Murashige and Skoog (MS) formulation. This basal medium is further supplemented with specific plant growth regulators—mainly cytokinins such as 6-benzylaminopurine (BAP) and kinetin, as well as auxins like indole-3-butyric acid (IBA)—at various concentrations.

The precise balance of these hormones plays a critical role in directing cellular responses, such as dedifferentiation, callus formation, or direct organogenesis through the development of adventitious shoots. Determining the optimal hormonal combination is essential for the successful initiation and multiplication of banana explants in vitro.

Table 1.

Effect of various NaOCl concentrations and four exposure times on disinfection of banana varieties meristem segments

Banana varieties	Treatment	NaOCl (1,0 %)				NaOCl (2,0 %)			
	Number of shoots	10				10			
	Duration	10 min	15 min	20 min	25 min control	10 min	15 min	20 min	25 min control
Miti Cavendish	Survival rate (%)	53,3	60,6	69,3	68,4	71,2	73,6	83,3	67,3
	Dead shoots (%)	11,4	15,3	10,4	21,3	14,5	12,4	7,4	15,3
	Infected with pathogens (%)	35,3	24,1	20,3	10,3	14,3	14,1	9,3	17,4
Super Dwarf	Survival rate (%)	58,5	61,4	64,6	65,8	73,3	85,2	71,1	63,3
	Dead shoots (%)	10,2	14,5	15,8	24,6	12,5	7,7	13,4	19,3
	Infected with pathogens (%)	31,3	24,1	19,6	9,6	14,2	7,1	15,6	17,4
Grand Naine	Survival rate (%)	38,5	53,4	62,6	64,8	61,1	76,2	79,1	57,3
	Dead shoots (%)	16,2	16,5	14,8	25,9	21,6	12,7	9,4	14,1
	Infected with pathogens (%)	43,3	30,1	22,6	9,3	17,3	11,1	11,5	19,7
Masak Xijayu	Survival rate (%)	69,3	71,5	78,3	74,1	61,3	68,6	66,3	63,8
	Dead shoots (%)	5,6	11,2	9,4	16,5	11,4	12,3	20,4	24,3
	Infected with pathogens (%)	25,1	17,3	12,3	9,4	17,3	19,1	13,3	11,9

In this study, we evaluated the effectiveness of different sodium hypochlorite (NaOCl) concentrations and exposure durations on the sterilization success of meristem segments in four banana (Musa spp.) varieties: Miti Cavendish, Super Dwarf, Grand Naine, and Masak Xijayu. The objective was to determine the optimal combination of concentration and exposure time that would ensure maximum explant survival while minimizing contamination and tissue death.

Two NaOCl concentrations (1.0% and 2.0%) were tested across four exposure durations (10, 15, 20, and 25 minutes). The performance of each treatment was assessed based on three criteria: survival rate (%), dead shoots (%), and contamination rate (% infected with pathogens).

Miti Cavendish showed the highest survival rate (83.3%) when treated with 2.0% NaOCl for 15 minutes. The lowest contamination rate (9.3%) was also observed under this condition, indicating it as the most effective treatment for this variety. However, increasing the exposure time beyond 20 minutes slightly reduced the survival rate due to increased tissue damage.

Super Dwarf responded best to 2.0% NaOCl for 10 minutes, with a survival rate of 85.2% and the lowest contamination rate (7.1%). Longer exposure times led to an increase in dead shoots, suggesting a higher sensitivity of this variety to prolonged sterilization.

Grand Naine had a relatively lower survival rate at 1.0% NaOCl treatments, but survival significantly improved with 2.0% NaOCl, especially at 15 minutes (79.1% survival and only 9.4% dead shoots). Contamination was also effectively reduced at this setting (11.5%).

Masak Xijayu performed well across most treatments, with the highest survival rate (78.3%) at 1.0% NaOCl for 20 minutes. Interestingly, this variety was relatively tolerant to longer exposures, although an increase in shoot mortality was noted at higher NaOCl concentration and exposure durations.

From the results, it can be concluded that both the concentration of NaOCl and the exposure time significantly influence the success of in vitro culture initiation. Optimal sterilization varied slightly by variety: For Miti Cavendish, the most effective treatment was 2.0% NaOCl for 15 minutes; For Super Dwarf, 2.0% for 10 minutes proved optimal; Grand Naine responded best to 2.0% for 15 minutes; Masak Xijayu showed good results with 1.0% for 20 minutes.

These findings provide a foundation for improving sterilization protocols in banana micropropagation, supporting higher survival rates and minimizing losses due to contamination and necrosis.

Table 2.

The effect of phytohormones in clonal micropropagation of banana varieties

Growth regulators mg/l		**MS (control) medium and growth medium**		**DKW medium and growth medium**		**MS enhanced nutrient medium and growth medium**		**WPM medium and growth medium**	
BAP	**IBA**	Number of regenera-ted branches	Length of regenera-ted branches	Number of regenera-ted branches	Length of regenera-ted branches	Number of regenera-ted branches	Length of regenera-ted branches	Number of regenera-ted branches	Length of regenera-ted branches
Miti Cavendish									
0	0,1	1:2	0,33	1:1	0,5	1:3	0,8	1:2	0,2
0,5	0,1	1:2	0,48	1:2	0,9	1:3	0,5	1:2	0,3

2,5	0,1	1:3	0,59	1:1	0,9	1:4	0,4	1:4	0,2
3	0,1	1:5	0,83	1:2	0,6	1:6	1,3	1:3	2,5
4	0,1	1:3	0,65	1:2	0,9	1:3	0,5	1:3	0,5
Super Dwarf									
0	0,1	1:2	0,38	1:1	0,5	1:	0,4	1:2	1,2
0,5	0,1	1:2	0,48	1:2	0,3	1:2	0,65	1:2	0,5
2,5	0,1	1:3	0,33	1:1	0,3	1:5	1.8	1:4	1,2
3	0,1	1:2	0,45	1:1	0,7	1:4	0,7	1:3	0,5
4	0,1	1:3	0,63	1:2	0,8	1:2	0,6	1:2	1,8
Grand Naine									
0	0,1	1:2	0,37	1:1	0,5	1:2	0,5	1:2	0,2
0,5	0,1	1:2	0,43	1:2	0,7	1:2	0,6	1:2	0,7
2,5	0,1	1:3	0,33	1:2	0,3	1:5	0,4	1:4	0,5
3	0,1	1:2	0,9	1:2	0,7	1:6	1,9	1:3	0,5
4	0,1	1:3	0,61	1:1	0,8	1:4	0,6	1:2	055
Masak Xijayu									
0	0,1	1:2	0,34	1:3	0,5	1:2	0,5	1:2	0,7
0,5	0,1	1:2	0,43	1:1	0,7	1:2	0,6	1:2	0,8
2,5	0,1	1:3	0,31	1:1	0,3	1:2	0,4	1:3	0,6
3,5	0,1	1:3	0,41	1:2	0,3	1:3	0,2	1:3	0,5
4	0,1	1:5	0,69	1:1	0,8	1:6	1,6	1:2	0,5

In this study, we investigated the role of phytohormones, specifically 6-Benzylaminopurine (BAP) and Indole-3-butyric acid (IBA), in the clonal micropropagation of four banana (Musa spp.) varieties: Miti Cavendish, Super Dwarf, Grand Naine, and Masak Xijayu. The aim was to determine the optimal concentration of growth regulators and culture media for inducing the highest rate of shoot regeneration and elongation.

BAP concentrations ranged from 0 to 4 mg/L, while IBA was kept constant at 0.1 mg/L. For each treatment combination, we recorded the average number of regenerated shoots (branching) and the average length of regenerated shoots.

1. Miti Cavendish: The most promising results were obtained with 3.0 mg/L BAP in enhanced MS medium, where the highest shoot multiplication ratio (1:6) and significant shoot elongation (1.3 cm) were recorded. The WPM medium also demonstrated exceptional elongation (2.5 cm) at the same hormone level, despite slightly fewer regenerated shoots (1:3). Overall, this variety responded well to higher concentrations of BAP, particularly in MS and enhanced MS media.

2. Super Dwarf: Maximum shoot elongation (1.8 cm) was observed in enhanced MS medium at 2.5 mg/L BAP, with a solid multiplication rate of 1:5. The WPM medium produced longer shoots (1.8 cm) at 4 mg/L BAP, although shoot proliferation was more modest (1:2). Interestingly, this variety showed a tendency for longer shoot elongation even at lower shoot

numbers, indicating it may be more suitable for direct shoot regeneration with proper elongation support.

3. Grand Naine: The enhanced MS medium at 3.0 mg/L BAP yielded both the highest shoot multiplication (1:6) and longest shoots (1.9 cm), suggesting a strong response to this treatment. MS and DKW media showed moderate regeneration rates (1:2–1:4) with shorter shoot lengths. This variety appeared to favor enhanced MS medium consistently, making it a reliable choice for mass propagation.

4. Masak Xijayu: The highest number of shoots (1:6) and elongation (1.6 cm) were again observed in enhanced MS medium at 4.0 mg/L BAP. The performance across MS and DKW was consistent but relatively modest in terms of shoot length. WPM performed adequately for elongation at lower BAP levels but didn't support high multiplication.

The data indicate a clear correlation between BAP concentration and both the number and length of regenerated banana shoots. Enhanced MS medium consistently supported better results across all four varieties, particularly when paired with BAP concentrations between 2.5 and 4.0 mg/L. This nutrient-rich medium likely provided the additional support needed for active cell division and elongation when combined with hormonal stimulation.

WPM medium, while not always optimal for shoot proliferation, showed potential for promoting elongation, particularly in Miti Cavendish and Super Dwarf, suggesting its usefulness during the shoot elongation or pre-acclimatization stage.

The varying responses among the different banana varieties also highlight the necessity of genotype-specific protocol optimization in tissue culture practices. While enhanced MS and moderate to high BAP levels showed overall effectiveness, individual tuning is essential for maximizing in vitro propagation success.

At a relatively lower concentration of 2.5 mg/L BAP, the majority of explants were observed to produce solitary shoots, rather than multiple shoot clusters. This finding is consistent with the observations of Shirani et al. (2010), who reported that lower concentrations of BAP tend to induce single shoot formation in banana tissue culture, likely due to limited cytokinin availability, which is essential for promoting active cell division and multiple shoot induction.

In addition, the application of Indole-3-butyric acid (IBA) has shown significant effects on root development during the acclimatization phase. Elhory et al. (2009) reported that the use of IBA resulted in the formation of robust and well-developed root systems, which subsequently improved the survival rate of plantlets upon transfer to soil. According to their findings, IBA-treated plantlets exhibited enhanced rooting efficiency and greater adaptability to ex vitro conditions, suggesting that IBA plays a crucial role not only in root induction but also in post-culture plantlet establishment and overall plant vigor.

Conclusions

This study successfully established initial protocols for the in vitro micropropagation of four banana cultivars under laboratory conditions in Uzbekistan. The results demonstrated that both the sterilization method and the composition of growth media significantly influenced the survival and regeneration capacity of banana explants.

Sodium hypochlorite (NaOCl) proved to be an effective surface sterilizing agent, with optimal concentrations and exposure times varying across cultivars. The most effective disinfection treatments minimized microbial contamination while maintaining high explant viability.

The use of phytohormones—particularly 6-Benzylaminopurine (BAP) and Indole-3-butyric acid (IBA)—played a critical role in shoot multiplication and elongation. Enhanced MS medium, in combination with 2.5–4.0 mg/L BAP and 0.1 mg/L IBA, consistently produced superior results across all varieties. Moreover, IBA significantly contributed to root development and improved plantlet survival during acclimatization.

These findings provide a solid foundation for the large-scale production of healthy, disease-free banana planting material in Uzbekistan. Further optimization and adaptation of these protocols to local conditions can support the sustainable expansion of banana cultivation in the country.

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