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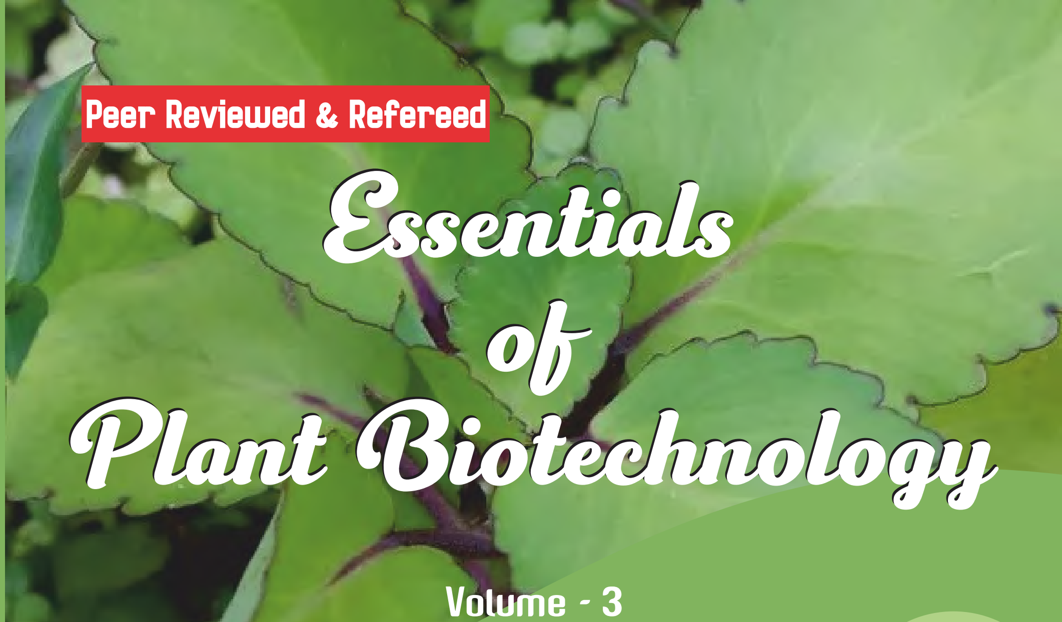

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Abstract

This chapter explores the challenges and limitations in the micropropagation of banana cultivars, while also providing future perspectives to enhance this vital process. Micropropagation, a technique used in plant tissue culture, is crucial for the rapid multiplication of disease-free, high-quality banana plants. However, it faces several challenges, including somaclonal variation, high production costs, and the need for skilled labour. The chapter delves into these issues, offering a comprehensive understanding of their implications on banana production. It also sheds light on the potential solutions to these challenges, focusing on recent advancements in biotechnology and their implications for banana micropropagation. Overall, this book chapter will enable future scientist and researcher in developing more cost effective and reliable technique and provide a comprehensive scenario to the available limitation and challenges in the mass production of this economically important fruit crop.

Keywords: Banana, micropropagation, challenges solutions, future perspectives

1. Introduction

For centuries, farmers have relied on suckers, the shoots emerging from the base of mature banana plants, to propagate new plants [1]. This method involves identifying a healthy mother plant, carefully sever a sucker with a well-developed root system, and replant it in a suitable location. However, beneath this apparent ease lie significant drawbacks the primary concern is the potential for disease transmission. Banana plants are susceptible to a variety of pathogens, including viruses like Banana bunchy top disease (BBTD) and bacterial wilt [2]. These microscopic invaders can silently reside within the mother plant, undetected by the naked eye and suckers have been known to harbour these pathogens. When such infected suckers are used for propagation, they inadvertently carry the disease to the next generation, perpetuating the cycle and ultimately impacting fruit quality and yield. Furthermore, traditional propagation is a slow and inefficient process. Sucker production is naturally limited, with a single mature plant typically generating only a handful of suckers per year. This sluggish multiplication rate presents a significant challenge for large-scale banana cultivation. Replacing diseased or unproductive plants becomes a time-consuming endeavour, hindering overall farm productivity. Additionally, the demand for bananas often fluctuates throughout the year due to seasonal trends or market demands [3]. However, sucker availability remains tied to the natural growth cycle of the banana plant, leading to potential gaps between supply and demand.

To combat these issues, *in vitro* propagation has emerged as a revolutionary technique in plant science that offers a powerful solution. This approach involves the controlled multiplication of plantlets under sterile laboratory conditions. By utilizing specialized tissue culture techniques, scientists and growers can generate large numbers of disease-free banana plantlets in a relatively short period of time.

2. Diseases affecting *Musa* spp.

Bananas (*Musa* spp.) are susceptible to several devastating diseases that significantly impact their production worldwide. Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *cubense* (Foc), is one of the most severe diseases, particularly the tropical race 4 (TR4) strain, which affects various banana cultivars including Cavendish, Gros Michel, and Silk bananas, leading to severe yield losses [4]. This strain has spread from Southeast Asia to regions such as Australia, the Middle East, Africa, and South America, including recent detections in Colombia, Peru, and Venezuela [5]. Another significant disease is the black leaf streak disease (BLSD), caused by *Pseudocercospora fijiensis*, which poses a major economic threat to banana cultivation in humid tropical regions. BLSD has been reported in various locations, including the Southwest Indian Ocean area and recently in Mauritius, where it was confirmed through molecular diagnostics and morphological observations [6]. Additionally, the fungal-nematode wilt complex, involving *Fusarium oxysporum* f. sp. *cubense* and *Radopholus similis*, is a serious threat, particularly to susceptible varieties like Nay Poovan. Integrated management approaches using bioagents, organic amendments, and chemicals have shown effectiveness in controlling this complex [7]. The spread of these diseases underscores the interconnectedness of global agricultural ecosystems and the need for vigilant monitoring and coordinated efforts to develop resistant banana varieties and implement biosecurity protocols to prevent further dissemination [5, 6]. The susceptibility of certain banana subgroups, such as Iholena, to Foc TR4 is particularly concerning for regions where these bananas are a staple food, highlighting the urgent need for comprehensive disease management strategies [4].

3. Benefits of micropropagation in *Musa* spp.

In vitro propagation offers numerous benefits for banana cultivation, significantly enhancing the efficiency and sustainability of banana production. One of the most critical advantages is the ability to produce disease-free plants and plantlets. By selecting healthy explants and employing sterile techniques, the risk of pathogen transmission is minimized, leading to healthier plants and improved fruit quality, which ultimately increases yields for farmers [8, 9]. Additionally, *in vitro* propagation allows for rapid multiplication of plantlets, far surpassing the slow and limited sucker production of traditional methods. This rapid multiplication rate enables farmers to quickly establish new plantations or replace diseased or unproductive plants, ensuring a consistent and sustainable supply of bananas [10, 11]. Furthermore, *in vitro* propagation is not dependent on seasonal fluctuations, as it allows for year-round production. By controlling the laboratory environment, optimal conditions for plantlet growth can be maintained throughout the year, ensuring a steady supply of healthy planting material regardless of climatic conditions [8, 12]. This capability is particularly beneficial for meeting market demands more effectively. Moreover, the optimization of plant growth regulators and surface sterilization techniques has further improved the efficiency and cost-effectiveness of *In vitro* propagation for different banana varieties [10, 12]. In conclusion, while traditional propagation methods have served for centuries, their limitations hinder large-scale banana cultivation. *In vitro* propagation, with its ability to generate disease-free plantlets rapidly and year-round, emerges as a powerful and innovative solution, paving the way for a more sustainable and productive future for banana cultivation.

4. Current international status on Banana micropropagation

Banana micropropagation has gained significant international traction as a vital technique for addressing the increasing global demand for bananas, driven by population growth and the need for food security. In Indonesia, biotechnology, including tissue culture techniques, is being leveraged to produce disease-free banana seedlings rapidly and on a large scale, which is crucial for diversifying staple foods and combating food crises [13]. In Nigeria, the temporary immersion bioreactor system (TIBs) has been identified as a cost-effective method for the rapid multiplication of banana plants, achieving high shoot multiplication rates and successful field establishment of morphologically normal and fertile plants [14]. Similarly, in India, an improved micropropagation protocol for the Grand Naine variety has been developed, focusing on combating oxidative stress during acclimatization, which has resulted in a high survival rate of 90% for *In vitro* plantlets [12]. The development of disease-free clones through micropropagation is also emphasized in the cultivation of the Poovan variety, with protocols showing high success rates in field establishment and morphological consistency with mother plants [15]. Globally, micropropagation is recognized as a crucial method for providing quality plants to meet nutritional and pharmaceutical needs, particularly in tropical and subtropical regions. While other fruit crops face challenges in tissue culture, banana remains the most commercially successful tissue culture product, with bioreactor-based micropropagation being the future direction for the industry [16]. Current international status on banana micropropagation underscores the importance of this technique in enhancing banana production, ensuring plant health, and meeting the growing global demand.

5. Current national status on banana micropropagation

Banana micropropagation in India has seen significant advancements, with various protocols being developed to enhance the efficiency and scalability of producing disease-free and high-yielding banana plantlets. The Grand Naine variety, widely cultivated in Uttar Pradesh, has been a focal point of micropropagation studies. Researchers have optimized the use of Murashige and Skoog (MS) media supplemented with specific concentrations of plant growth regulators like BAP and IAA for shoot proliferation and IBA for rooting, achieving high success rates in generating healthy plantlets [17]. Similarly, the Poovan variety has shown promising results with the use of TDZ and BAP for shoot multiplication and IBA for root induction, leading to a high establishment rate of regenerated plantlets in the field [15]. The importance of micropropagation is further underscored by its role in producing banana propagules for different genomic groups, with studies demonstrating the effective use of BAP and substrates like sawdust and vermicompost to induce sucker formation in various banana genotypes [18]. Additionally, commercial-scale micropropagation efforts have focused on making the process cost-effective for farmers by using economical cytokinins like BAP and effective auxins like IAA, particularly for cultivars such as Grand Naine, Monthan, and Red Banana [19]. The broader context of micropropagation in India highlights its critical role in meeting the nutritional and pharmaceutical needs of a growing population, with banana being the most commercially utilized tissue culture product. The use of bioreactors is suggested as a future direction to further enhance the efficiency and scalability of banana micropropagation [16]. Overall, the national status of banana micropropagation in India is robust, with ongoing research and development aimed at optimizing protocols for various cultivars to ensure high-quality, disease-free plantlets for commercial cultivation.

6. Challenges and solutions: Troubleshooting in micropropagation of *Musa* spp.

The world of *in vitro* banana propagation, while fascinating, is not without its challenges. Even with meticulous protocols, researchers and growers can encounter hurdles along the way. Despite stringent aseptic techniques, contamination by bacteria, fungi, or other microorganisms can occur. Signs of contamination can manifest as discoloration of the culture medium, browning of explants, or fuzzy/moldy growth on the explants or culture vessel.

To combat the challenge of contamination, reevaluating and reinforcing aseptic practices within the laminar Air flow chamber becomes necessary. Further, incorporating fungicides or antibiotics into the medium at recommended concentrations can also help control specific contaminants. In majority of the cases, explants have been found to exhibit browning or necrosis (cell death) after inoculation. This may be due to mechanical damage during excision, harsh sterilization or simply the composition of the medium being used. These issues with the explant can be resolve by proper explant examination, monitoring of sterilization phase and selection of best basal medium supplementation. Further, addition of antioxidant such as citric acid or ascorbic acid has been found to alleviate browning.

7. Future perspectives in banana micropropagation

The future perspectives in banana micropropagation include the development of cost-effective media, optimization of protocols for

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recalcitrant cultivars, genetic improvement through biotechnological interventions, and the use of advanced techniques like somatic embryogenesis and temporary immersion bioreactor systems.

Researchers are focusing on developing cost-effective culture media and optimizing micropropagation protocols for large-scale propagation of banana cultivars. This involves the use of locally available, low-cost ingredients and the optimization of plant growth regulators and other media components to reduce production costs while maintaining high multiplication rates and plant quality.

Genetic improvement of banana through biotechnological interventions, such as genetic modification and genome editing, holds great potential for developing cultivars with desirable traits like disease resistance, abiotic stress tolerance, and enhanced nutritional quality. The use of techniques like CRISPR/Cas9 allows for the targeted modification of genes responsible for these traits, enabling the development of improved banana varieties.

Somatic embryogenesis is a promising technique for the regeneration of banana plants from somatic cells. It offers advantages like high multiplication rates, genetic stability, and potential for cryopreservation. Ongoing research aims to optimize protocols for efficient induction, development, and maturation of somatic embryos for large-scale propagation of banana cultivars.

Temporary immersion bioreactor systems, such as RITA® and BIG®, have shown promising results for the large-scale multiplication of banana plantlets. These systems allow for better control of the culture environment, reduced labor costs, and higher multiplication rates compared to conventional micropropagation methods. Future research will focus on optimizing these systems for specific banana cultivars and scaling up production.

Improving the acclimatization and field performance of micropropagated banana plantlets is another area of focus. Researchers are working on developing efficient hardening protocols, optimizing the composition of potting media, and evaluating the agronomic performance of micropropagated plants under field conditions to ensure the successful establishment and productivity of banana plantations.

Fig 1: A-B: Banana suckers (young shoots) traditionally used to propagate Banana trees

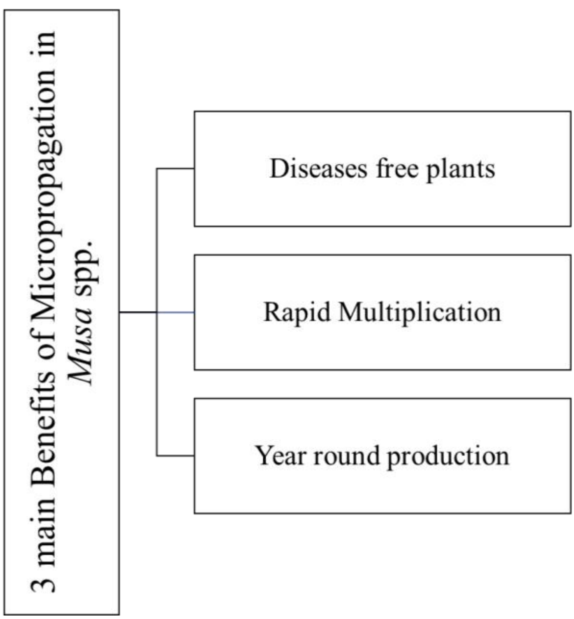

Fig 2: Benefits of micropropagation in *Musa* spp.

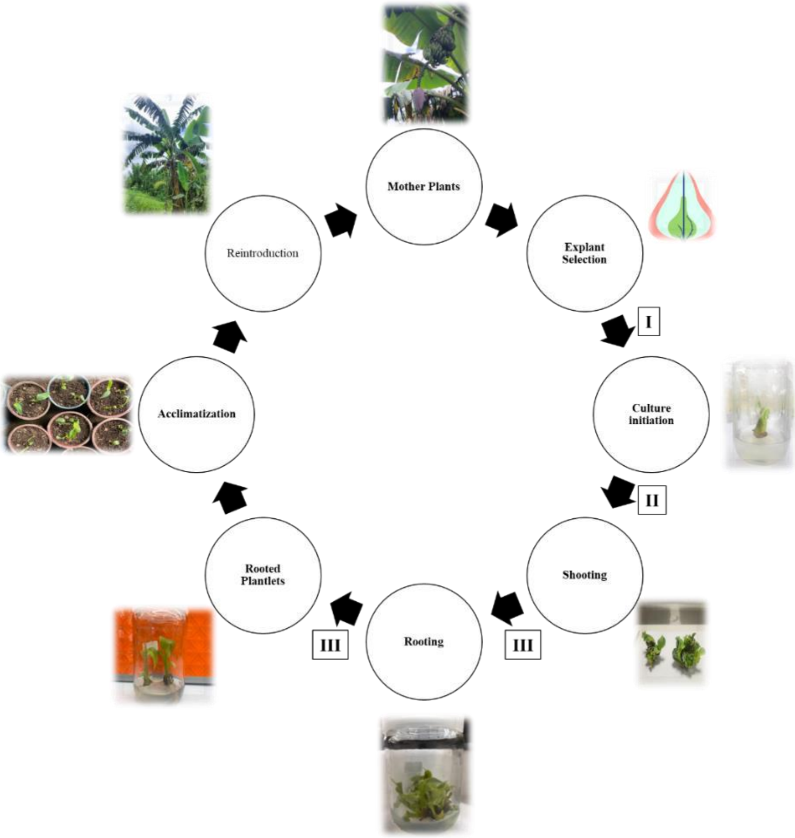

Fig 3: General steps in the micropropagation of *Musa* spp.

8. Conclusion

Micropropagation of banana cultivars presents a promising yet challenging avenue for the large-scale production of high-quality planting materials. The technique is particularly advantageous for banana, a staple crop in many tropical and subtropical regions, due to its ability to produce a large number of true-to-type plants in a limited space and time, independent of climatic conditions. However, the process is fraught with several technical, biological, and physiological challenges. For instance, different banana varieties require specific media compositions for optimal growth, as seen with the Robusta and Nendran varieties, which necessitate tailored plant growth regulator regimes to achieve desirable shoot multiplication and rooting. Additionally, the presence of diseases such as Moko, caused by Ralstonia solanacearum, underscores the need for disease-free propagules, making *In Vitro* propagation a viable solution for producing certified quality plantain seeds. Despite these advantages, micropropagation faces obstacles such as contamination, somaclonal variations, and other physiological disorders, which can be mitigated by adhering to species-specific protocols and improving laboratory infrastructure. Moreover, genetic fidelity remains a critical concern, but studies using molecular markers like SSR and ISSR have confirmed the clonal uniformity of micropropagated plants, ensuring their genetic identity matches that of the mother plant. Future perspectives in banana micropropagation include the upscaling of existing protocols, especially for recalcitrant species, and the adoption of bioreactor systems to enhance efficiency and reduce costs. Addressing these challenges through continued research and technological advancements will be crucial for the sustainable and commercial success of banana micropropagation, ultimately contributing to food security and economic stability in banana-producing regions.

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